Panel on Neuro-glia Mechanisms of Chronic Pain

A Retrospective and the Role of Microglia & Astrocytes in Chronic Pain Mechanisms

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Microglia and pain: 1043
Astrocytes and pain: 769
Satellite glial cells and pain: 163
Oligodendrocytes: a few?

Glia and pain: 1,717
Neuron and pain: 22,957
Glia have different activation states in chronic pain

1. Gliosis: Changes of glial markers and morphology

2. UP-regulation of glial receptors and channels

3. Intracellular signaling: Phosphorylation of MAP kinases

4. Production of glial mediators

Release of glial mediators (pain mediators)

Interaction with neurons and central sensitization

Chronic pain

Ji, Berta, and Nedergaard, *Pain*, 2013
I. Microglia and chronic pain

II. Astroglia and chronic pain

III. Gliopathy drives chronic pain

IV. Clinical significance & future directions
Macrophages of the CNS

- Major effectors of the CNS innate response
- Express CD11b, IBA-1, CX3CR1
- Major source of cytokines in the CNS (TNF-α, IL-1β, IL-18)
- Produce growth factor BDNF
- Microgliosis (reactive) after nerve injury
- Pathogenesis of neuropathic pain
- Acute inflammatory pain?
Time-dependent microglia activation after CCI

Mechanical Allodynia

Heat Hyperalgesia

Days after nerve injury (CCI)
Phosphorylation of p38 MAPK after SNI

p-p38 / CD11b (OX-42)

Numerous animal studies in different models (Intrathecal injection)

Some human studies (Systematic route) CNS effects?
Inflammatory pain
Postoperative pain

**Activators**
- ATP, CSF-1
- CX3CL1, CCL21, CCL2
- Caspase-6, MMP-9, cathepsin S

**Receptors**
- P2X4, P2X7, P2Y12, CSF1R
- CX3CR1, CCR2
- TLR4, TLR2

**Microglial mediators**
- TNF
- IL-1β
- IL-18
- BDNF

**Tissue injury**
- Surgery

**Nerve injury**
- Cancer

**Chronic opioid**

**Neuropathic pain**
- Opioid tolerance
- Hyperalgesia

**Cancer pain**

**Postoperative pain**

**Inflammatory pain**

**Intracellular signaling in microglia**

**P-p38**
Single-cell analysis for microglial gene expression

Microglial cell collection from spinal cord slice of Cx3cr1-GFP mice

Single cell PCR

Microglia are primary source of TNF in the spinal cord.

Berta et al., JCI, 2014
Caspase-6 releases TNF-α from microglia to modulate synaptic transmission.

![Graph showing TNF-α release](image)

- TNF-α
- IL-6
- IL-1β

![Diagram of the nervous system](image)

- DRG neuron primary afferent
- Microglia
- Excitatory interneuron
- Dorsal horn
- Pre-synaptic
- Post-synaptic
- Microglia
- Astrocyte
- TrPV1
- Glu
- TNF-α
- CASP6

![Patch clamp recording](image)

- TNF-α (10 ng/ml)
- Capsaicin (1 µM)
- 20 pA
- 1 min

Berta et al., *JCI*, 2014
Astrocytes

- Most abundant cells in the CNS
- Express GFAP, GLT1, Cx43, ALDH1-L1
- Provide structural and tropical support for neurons
- Control synapse formation & insulate synapses
- Interact with blood vessels
- Form networks via gap junctions
- Form glymphatic system to clear toxins
- Maintain glutamate and K⁺ homeostasis
- Release gliotransmitters such as ATP, glutamate, cytokines, chemokines
Persistent activation of astrocytes in neuropathic pain

CCI, 10 days

CCI, 21 days

GFAP

Injury side
Astrocytic Cx43 maintains late-phase neuropathic pain

Chen et al., Brain, 2014

CBX: carbenoxolone
Gap junction blocker

Peptide inhibitor of Cx43

Cx43 / GFAP
Cx43 modulates synaptic transmission after CCI 3 weeks

Patch clamp recording in I1o neurons
Cx43 maintains neuropathic pain
via CXCL1 release from astrocytes
Astrocytes produce chemokines via JNK

Chemokines (Astrocytes)
- CCL2/MCP-1
- CXCL1/KC

Chemokine receptors (Neurons)
- CCR2
- CXCR2

Gao and Ji, Neurotherapeutics, 2010
Different types of spinal cord astrocytes

CCI injury

GFAP

Merge GFAP / Aldh1

Aldh1-EGFP

ALDH1-L1 (Aldehyde dehydrogenase 1, L1)
**Gliopathy in chronic pain: Astrocyte dysregulation**

- **Glutamate homeostasis**
  - GLT1, GLAST
  - Hyper-excitability

- **Potassium homeostasis**
  - Kv4.1, Kv5.1
  - Hyper-excitability

- **Water homeostasis**
  - AQP4 dysfunction
  - Edema

- **Chemokines**
  - (CXCL1, CCL2)

- **Long-range signaling**
  - Cx43 dysfunction

- **Paracrine signaling**
  - ATP, glutamate

Modified from Verkhratsky et al., *ASN Neuro*, 2012

Ji et al., *Pain*, 2013
Targeting glial cells for chronic pain

- Chronic pain is a result of “gliopathy”.
- Microglia and astrocytes are important players in chronic pain development and maintenance.
- Glia modulate pain via “neuro-glia interactions
- Glia-produced mediators (cytokines and chemokines) are neuromodulators and can powerfully modulate synaptic transmission.

Strategies

- Cytokine inhibitors (TNF, IL-1β, IL-6)
- Anti-inflammatory cytokines IL-10, TGF-β
- Chemokine inhibitors (CXCL1/CCR2, CCL2/CXCR2)
- MAPK inhibitors (p38, JNK, ERK)
- Proteases inhibitors (MMP-9, MMP-2, CASP6, Cathepsin S)
- Cx43, P2X7, TLR4 inhibitors
- Cell therapy (bone marrow stem cells)
What can we learn from cancer therapy?

Cancer

- Chemotherapy
- Immune therapy
- Combination therapy

Chronic Pain

- Neuron-targeting therapy
- Glia-targeting therapy
- Combination therapy

Strategies

- Multiple drugs to target neurons and glia separately
- One drug that can target both neurons and glia
Biosynthesis of resolvins and neuroprotectin
(Anti-inflammatory and pro-resolution mediators)

**EPA**
- Aspirin:COX-2
- **P450**
- **microbial**

**DHA**
- Aspirin:COX-2
- **LOX**

**17R-Resolvin D Series**
**Acetylated COX-2**

**17S-Resolvin D Series**
**Lipoxygenase mechanism**

**Epoxidation**

**LOX**

**Neuroprotectin D1 (NPD1)**

**18R-H(p)EPE**

**RvE1**

**RvD1**

**RvD2**
Neuropathic pain relief by neuroprotectin D1 (NPD1)

Xu et al., *Annals of Neurology*, 2013

![Chemical structure of NPD1](image1)

**Mechanical Allodynia**

- **Sham**
- **Vehicle**
- **NPD1, 300 ng**

<table>
<thead>
<tr>
<th>Time after CCI</th>
<th>Paw withdrawal threshold (g)</th>
</tr>
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<tbody>
<tr>
<td>BL</td>
<td>1.6</td>
</tr>
<tr>
<td>3 d</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>7 d</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>14 d</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>21 d</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>28 d</td>
<td>1.2 ± 0.2</td>
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**Mechanical Allodynia**

- **Vehicle**
- **NPD1, 20 ng**
- **NPD1, 100 ng**
- **NPD1, 500 ng**
- **DHA, 100 μg**

<table>
<thead>
<tr>
<th>Time after i.t. injection</th>
<th>Paw withdrawal threshold (g)</th>
</tr>
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<tbody>
<tr>
<td>BL</td>
<td>1.6</td>
</tr>
<tr>
<td>CCI 2 w</td>
<td>1.0</td>
</tr>
<tr>
<td>0.5 h</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>1 h</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>3 h</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>24 h</td>
<td>1.4 ± 0.2</td>
</tr>
</tbody>
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**Normalized PWT (% of BL)**

- **NPD1**
- **Gabapentin**

![Graph showing normalized PWT (%) of BL](image2)

Xu et al., *Annals of Neurology*, 2013
Neuroprotectin D1 (NPD1) modulates glial and neuronal activities after nerve injury

Neuroinflammation in the spinal cord after CCI

Long-term potentiation in the spinal cord

Xu et al., Annals of Neurology, 2013
Neuroprotectin D1 (NPD1) based therapies

Glia activation and Neuroinflammation

- TNF-α
- IL-1β
- CCL2
- CXCL1
- BDNF

Maladaptive synaptic plasticity

Chronic pain

NPD1

- Anti-inflammation
- Pro-resolution
- Very safe
- Inhibit TRPV1 function
- Inhibit neural plasticity
- Inhibit glial activation
- Promote regeneration
- Protect neurons
- Limitation: unstable

- **NPD1**: Prevention of nerve trauma and chemotherapy induced neuropathic pain
- **Small molecule receptor agonist of NPD1R**: Treatment of established chronic pain
Future directions

- Identify new markers for microglia and astrocytes
- Unbiased approaches to screen mediators produced by glial cells
- Further investigations into neuro-glia interactions
- Other glial types (satellite glia, oligodendrocytes)
- Selective tools to target glia (chemical genetics, DREADD)
- Sex-dependent glial signaling
- Age-dependent glial signaling
- Glia activation in human brain
- Glymphatic system in chronic pain
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