Growth factors, cytokines, chemokines and pain

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February 23, 2015
Readings:


1. Growth factors in pain signaling
2. Immune signaling and pain
   Peripheral effects
   Central effects
   - Cells that contribute to immune signaling
   - Mediators of immune signaling
     - Cytokines
     - Chemokines
     - TLR system
3. Opioid signaling and central immune signaling
4. Strategies to manipulate neuroimmune signaling to treat pain
Neurotrophic Growth Factors

Neurotrophins - NGF, BDNF, NT3 and NT4

Retrograde growth factor signaling during development controls the survival and targeting of developing DRG sensory neuron subtypes.

Nerve Growth Factor –

- Required for development of nociceptor neurons.
- Highly expressed in damaged or inflamed tissues
- Genetic mutations in NGF or trkA cause congenital insensitivity to pain
- Injection of NGF causes pain
- Facilitates pain transmission by nociceptor neurons
Blocking NGF largely blocks the effects of inflammation (hyperalgesia, spontaneous activity) on sensory nerve function.
NGF is elevated in: cystitis, arthritis, skin inflammation, pancreatitis and by CFA, IL1β or TNF injection.

Modulates expression/release of neuropeptides, TRP ion channels, sodium channels (Nav1.8)...

Signaling pathways important in sensitization of TRPV1 by TrkA.

NGF binding/trk phosphorylation leads to src phosphorylation of TRPV1 Y200, promoting insertion of TRPV1 into the cell membrane.

NGF stimulates BDNF expression

BDNF immunoreactivity 24h post intrathecal NGF.

Increase in NTs in peripheral tissues and Schwann cells

BDNF/trkB $\rightarrow$ Ca$^{2+}$ $\rightarrow$ changes in ion channel state/activity
Anti-NGF clinical trials: Tanezumab (monoclonal to NGF, Pfizer/Lilly)

Osteoarthritis – phase 1-3 trials. Improved function and reduced pain. 32 week trial: 16 of 690 patients developed worsening OA, 4 patients had joint replacements (knee/hip).

Chronic low backpain – phase 2. Significant improvement over Naproxen group.

Interstitial cystitis – phase 2. Analgesic effect was superior to placebo.

Chronic prostatitis and cancer – phase 2, results not yet reported.

Adverse Effects: most common is abnormal sensation.

55-68% in OA

30% in LBP

47% in IC

Comparable to placebo groups in most trials.
Glial cell line-derived neurotrophic growth factors - GDNF, neurturin, artemin, persephin

Anti-artemin blocks cystitis-induced bladder hyperalgesia and central pERK activation.

Malin et al. (2006) J Neurosci
Cells and molecules that mediate neuroimmune signaling
Adaptive or acquired immunity - Ab and lymphocyte responses to antigens.

Cells of the immune system interface between neural and non-neural components of the nervous system.

Innate immunity – generalized immune response to infectious agents, cell debris. Activation initiates a cascade of cytokine synthesis.

Model of Immune Responses: Speed and Specificity
Evolution of Chronic Pain as a Neuro-Immune Disorder

- Peripheral nerve injury (trauma, disease or drug induced) causes an immune response that attracts immune cells that clear damaged tissue & pathogens and promote repair (1970’s).

- Growth factors, cytokines and chemokines mediate this inflammatory process and sensitize nociceptors (increasing ion channel/receptor/synaptic activity). 1992 - An inflammatory soup of BK, serotonin, PGE2 and histamine shown to excite nerve fibers.

- Pain is a symptom of the inflammatory process and normally fades as inflammation resolves.

- In some cases, even though the injury appears healed, abnormal hyperactivity in pain signaling pathways remained, producing chronic pain. Treatments to reduce neuronal activity often were ineffective in blocking this pain.
This led to studies (1990’s) on the involvement of non-neuronal cells in chronic pain signaling and the understanding that the inflammatory process and interactions between neurons, immune cells and immune-like glial cells are important in pain resolution.

Sciatic nerve after transection injury – perineural sheath compromised.

Inflammatory soup bathes nerve fibers → spontaneous activity.

Chronic (neuropathic) pain management → suppress neuronal activity.

- Could modulating the immune response to nerve injury and targeting non-neuronal cells modify the disease as well as neurobiological changes?
Peripheral tissues: proinflammatory immune signaling contributes to the initiation and maintenance of pain states.

In peripheral nerves, injury provokes reactions in Schwann, immune and satellite cells that contribute to acute and chronic sensitization.

In DRG, neurons and resident macrophages release immune mediators that recruit macrophages that sustains an inflammatory milieu for months.
In the spinal cord, neurons and non-neural cells contribute to sensitization and repair.

Neurons respond to immune regulators, express and release cytokines, chemokines, TLRs linked to proinflammatory pathways.
Astrocytes  Support neurons and endothelial cells that form blood-brain barrier, provide nutrients
Regulate blood flow; support repair following injury
Propagate intercellular Ca\textsuperscript{2+} waves through coupled gap junction networks
Respond to neurotransmitters; regulate uptake/release of glutamate
Express potassium channels; regulate uptake/release K\textsuperscript{+}
Synthesize and release cytokines, chemokines

GFAP labeling
The astrocyte, presynaptic and postsynaptic terminals and their ability to integrate synaptic activity and release neuromodulators is termed the tripartite synapse.

Astrocytes respond (elevate Ca$^{2+}$) to most neurotransmitters and can release glutamate, ATP, NO, PG which in turn modulate neuronal excitability.

A single astrocyte may wrap 140,000 synapses and 4-6 neuronal somata.
Microglia

Resident CNS macrophages, ~15% of CNS cells

Surveillance function, provide first and main immune defense

Phagocytic, remove apoptotic cells, maintain & eliminate synapses

Activated by injury, glutamate agonists, pro-inflam cytokines

Synthesize and release pro- and anti-inflammatory cytokines
Glia become ‘activated’ and proliferate in response to injury.

**Table 1**
Distinct reaction of microglia, astrocytes, and satellite glial cells (SGCs) in different pain conditions, as examined by upregulation of the glial markers IBA1, CD11b, and glial fibrillary acidic protein (GFAP).

<table>
<thead>
<tr>
<th>Pain conditions</th>
<th>Microglia</th>
<th>Astrocytes</th>
<th>SGCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerve injury</td>
<td>←/↑</td>
<td>←/↑</td>
<td></td>
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<tr>
<td>Spinal cord injury</td>
<td>←/↑</td>
<td>←/↑</td>
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<tr>
<td>Paw incision</td>
<td>←</td>
<td>←</td>
<td>↑</td>
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<tr>
<td>Inflammation</td>
<td>←/↑</td>
<td>↑</td>
<td></td>
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<tr>
<td>Joint arthritis</td>
<td>←/↑</td>
<td>↑</td>
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<tr>
<td>Bone cancer</td>
<td>←/↑</td>
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<tr>
<td>Skin cancer</td>
<td>←</td>
<td>←</td>
<td>↑</td>
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<tr>
<td>Chemotherapy</td>
<td>←/↑</td>
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<tr>
<td>Diabetes</td>
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<tr>
<td>HIV neuropathy</td>
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<tr>
<td>Chronic opioid</td>
<td>←</td>
<td>←</td>
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<tr>
<td>Acute opioid</td>
<td>←</td>
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</tbody>
</table>

Ji et al., (2013)

Activation of microglia 3d post nerve injury.
BUT what does glial activation really reflect?

Do reactive glia aid in recovery from injury or impair recovery?

Does activation always cause pain; does it function to block pain?

NEED TO KNOW

What is the spatial and temporal pattern of the inflammatory response?

What growth factors/cytokines/chemokines are made and how do they contribute to inflammatory/neuropathic pain?

What cells synthesize which cytokines? Are they pro- or anti-inflammatory?
Cytokines

- Small cell-signaling proteins that modulate cell-cell interactions and migration through receptor binding.
- Maintain homeostasis, respond to stress and immune challenges.
- Are secreted by glia, immune cells and neurons.

Classes of cytokines:
- Interleukins (IL) – JAK-STATs
- Chemokines
- Interferons (IFN) - JAK-STATs
- Colony-stimulating factors - JAK-STATs
- Tumor necrosis factors (TNF) – NF-kB, MAPK pathways
- Transforming growth factors (TGFbetas) – Smads
Cytokines IL-1β, IL-6 and TNF are the most well-studied proinflammatory mediators.

Increased in inflamed or injured tissue; leads to activation of neurons directly or indirectly by stimulating release of other mediators, i.e., prostaglandins.

Local/systemic injection of each causes pain. Compound-specific antibodies block pain.

**IL-1β** – one of the first cytokines linked to injury-related pain. Made by microglia and neurons in spinal cord. Can directly modulate neuron properties through changes in ion channel properties.

24h exposure to IL-1β potentiates voltage dependent Na⁺ current in trigeminal neurons.
IL-6 – has pro- and anti-inflammatory effects. Stimulates acute phase immune response to trauma.

Sensitizes TRPV1 and provokes CGRP release via JAK activation.

Can be inhibitory through effects on TNF, IL1b
TNFα (cachectin) – role in peripheral and central sensitization. Rapid increase in inflamed or injured peripheral tissue.

- Direct application to nerve induces ectopic activity (TNFR → MAPK → Na⁺ channel phosphoryl).

- Sensitizes nociceptors to heat-evoked release of CGRP in s.c.

TNF has both immediate and long term effects.
Chemokines

- Family of small (8-10 kDa) cytokines (~50) that signal through GPCRs (~18).
- Induce directed chemotaxis (chemotactic cytokines).
- Have homeostatic and proinflammatory roles
  - Control migration of cells in development and during tissue maintenance.
  - Attract leukocytes (monocytes, neutrophils) to site of infection or injury.
  - Produce pain when injected.
- Neurons and glia produce and respond to chemokines.
Four groups of chemokines based on the spacing of their first two cysteine residues.

C chemokines – 2 known (lymphotactins); attract T cell precursors to thymus.

CC chemokine ligands – CCL-1 → CCL-28. Induce migration of monocytes, NK cells and dendritic cells. CCL2 = MCP1, monocyte chemotactic protein 1.

CXC chemokines – 17 members; Induce migration of neutrophils or lymphocytes. Bind to CXC receptors (7 are known).

CX3C chemokine – aka fractalkine (CX3CL1). Is secreted and tethered to surface of cell expressing it; is an attractant and adhesion molecule.
While proinflammatory cytokines are expressed early on and contribute to the genesis of chronic pain, chemokines are expressed later and may act as triggers to convert acute pain to chronic pain.

Prolonged chemokine/R expression in sensory ganglia may contribute to neuropathic pain syndromes.
Chemokines facilitate reciprocal signaling between neurons and non-neuronal cells;

Eg.- DRG neuron to spinal cord microglial signaling.

DRG neurons express fractalkine (CX3CL1). Fractalkine binds CX3CR1 on microglia in either a membrane ‘tethered’ or lysosomal cathepsin S protease-cleaved released state. (Cath-S is made by activated microglia.)

PAIN → Cath-S releases tethered fractalkine from neurons, which then activates the microglial expressed fractalkine GPCR (CX3CR1), causing upregulation and release of inflammatory mediators (TNF, IL1β, IL6) and amplification of ongoing pain.
Cathepsin S expression in DH microglia after peripheral nerve injury.
Chemokine upregulation (e.g., CXCR3) in immune-mediated neuropathies, e.g., demyelinating diseases of the CNS (multiple sclerosis) and PNS (GB).

Guillain-Barre’ syndrome – increases in chemokines may underlie disease-associated pain.

Human sural nerve; perivascular infiltrate in GBS.

T cells (CD3+ ) are CXCR3 positive.

Kieseier B C et al. Brain 2002;125:823-834
Adaptive or acquired immunity - Ab and lymphocyte responses to antigens.

Innate immunity – generalized immune response to infectious agents, cell debris. Activation initiates a cascade of cytokine synthesis.
Complement System and Pain Signaling

- 11 plasma proteins made by liver; circulate in inactive forms until the “complement cascade” is initiated.
- Part of the innate immune response activated to eliminate microbes.
- Activation culminates in formation of the membrane attack complex (MAC), leukocyte attraction and ingestion of pathogens by phagocytes.
Inappropriate activation of complement occurs in demyelinating neuropathies (multiple sclerosis, Guillain-Barre syndrome).

Other links to nerve injury, inflammatory a incisional pain.

*Anaphylatoxins* such as C5a affect migration and adhesion of immune cells to injured nerve, DRGs and spinal cord.

Intraplantar or intrathecal injection of C5a elicits heat and mechanical hyperalgesia whereas C5a antagonists diminish pain.

Nerve injury induces activated complement expression in spinal cord and DRG (satellite cell glia, macrophages).

In peripheral nerves complement facilitates myelin clearance; in the SC may elicit pain by activating microglia that express C5aR.

*complement peptides that mediate inflammation.
Critical to central immune signaling are Toll-like receptors (TLRs)

- TLRs are pattern recognition receptors; sense exogenous / endogenous damage or danger signals associated with microbial pathogens or cellular stress.

- Are link between innate immune system and the CNS.
TLRs initiate pain-related (proinflammatory) cytokine activation.

TLRs - selectively expressed on macros, dendritic cells, DRG cells, microglia, astrocytes, Schwann cells, leukocytes, keratinocytes, etc.

PAMPs & DAMPs (fibronectin, defensins HSPs) activate TLR signaling.

TLR activation triggers the innate immune response, leading to cytokine cascade.

TLR activation in astrocytes
TLR activation in microglia
TLR activation in neurons suggests a direct link between PAMP/DAMP TLR activation, bypassing immune cells.

TLR mediated cytokine release by astrocytes/microglia can also increase neuronal excitability and synaptic strength through effects on AMPA/NMDA receptors, K⁺/Na⁺ and GABAergic signaling.
### Proinflammatory Mediators released via TLR activation

<table>
<thead>
<tr>
<th>TLR1</th>
<th>MCP-1, ROS, NO, CXCL8, IL-6, IL-12p40</th>
<th>TNF-α, IL-1β, iNOS, COX-2, NO, CCL2, CXCL2</th>
<th>Expressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2</td>
<td>MCP-1, ROS, TNF-α, IL-6, CCL3, NO, IL-1α, IL-1β</td>
<td>IL-1β, IL-6, TNF-α, IL-12, NO</td>
<td>PGE₂, RANTES, CXCL10, IL-1α, IL-1β</td>
</tr>
<tr>
<td>TLR3</td>
<td>MCP-1, TNF-α, IL-6, IL-1α, IL-1β, NO, IFN-β, iNOS</td>
<td>IL-1α, IL-6, CCL3, CXCL2, TNF-α, IL-18, iNOS, NO</td>
<td>CGRP, TNF-α, IL-1β</td>
</tr>
<tr>
<td>TLR4</td>
<td>IL-6</td>
<td></td>
<td>Expressed</td>
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<tr>
<td>TLR5</td>
<td>IL-6</td>
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<tr>
<td>TLR6</td>
<td>IL-6</td>
<td></td>
<td>Expressed</td>
</tr>
<tr>
<td>TLR7</td>
<td>IL-6, IL-1α, TNF-α, IL-5, IL-13</td>
<td>IL-6, TNF-α, IL-1α, IL-12, IL-13, IL-15</td>
<td>PGE₂, RANTES, CXCL10, IL-1α, IL-1β</td>
</tr>
<tr>
<td>TLR9</td>
<td>MCP-1, ROS, IL-6, TNF-α</td>
<td>IL-6, TNF-α, IL-5, IL-13, IL-15, NO</td>
<td>PGE₂, RANTES, CXCL10, IL-1α, IL-1β</td>
</tr>
</tbody>
</table>

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1. **Glial reaction: changes of glial markers and morphology**
2. **Regulation of receptors, channels, and transporters in glia**
3. **Phosphorylation of MAP kinase signaling pathways in glia**
4. **Production of cytokines, chemokines, growth factors in glia**
   - Release of glial mediators
   - Interaction with neurons: central and peripheral sensitization
   - Persistent pain
Opioids and Neuroimmune Signaling

Limited opioid efficacy, paradoxical hyperalgesia, abuse potential and side effects are key problems. Does opioid-induced central immune signaling contribute to these adverse effects?

- Astrocyte, microglia and oligodendrocytes express delta, kappa and mu opioid receptors.

- Both nerve injury and morphine cause glial hypertrophy, increased spinal expression of IL-1b, IL-6, TNF and chemokines.
Increased microglia and astrocyte activation by chronic morphine treatment in rats. The morphine-induced increase in GFAP correlates with loss of analgesic effect (tolerance).

Raghavendra et al, 2002)

10mg/kg twice daily for 5d. Level L5 of spinal cord.
Propentofylline (PPF- adenosine reuptake inhibition)

Reverses reactive gliosis following nerve injury.

Dose-dependently reduces morphine-induced hyperalgesia:
Subcu morphine (rats) for 5d; PPF given 1x daily with morphine.

Tail flick Latency (s)

Paw pressure Latency (g)

Measures made 16h post last morphine/saline injection.

Mechanism?? Nerve injury and chronic morphine cause downregulation of glutamate transporter (GLT1) → elevation in extracellular glutamate → pain. PPF enhances GLT-1 expression.
Opioid-induced central immune signaling in astrocytes.

Opioid-induced central immune signaling in astrocytes.
Opioid-induced central immune signaling in microglia.

# The Neuroimmune Interface: Pain Control

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Cellular targets</th>
<th>Mechanism of action</th>
<th>Clinical use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>Microglia</td>
<td>Disrupts TLR4 signalling by binding to MD2</td>
<td>Depression</td>
</tr>
</tbody>
</table>
| ATL313 (Santen Pharmaceutical)| Microglia and astrocytes             | * Adenosine A2A receptor agonist  
* PKA and PKC activation                        | NA                                              |
| BAY 60–6583 (Bayer HealthCare)| Microglia and astrocytes             | Adenosine A2B receptor agonist                                                      | NA                                              |
| Ceftriaxone                   | Astrocytes                           | Increases EAAT2 expression by inhibiting NF-κB activity                              | * Bacterial meningitis  
* Lyme disease                                     |
| Dilmapimod                   | Microglia                            | Selective p38 MAPK inhibition                                                       | NA                                              |
| FP-1                         | Microglia                            | TLR4 antagonist                                                                     | NA                                              |
| Glatiramer acetate            | T cells                              | Promotes generation of anti-inflammatory T cell phenotypes                          | Multiple sclerosis                                |
| Ibudilast (MN-166; MediciNova)| Microglia, astrocytes and T cells    | Non-selective phosphodiesterase inhibitor                                            | * Asthma  
* Post-stroke dizziness                          |
| Methotrexate                  | T cells                              | Suppresses expression of cell adhesion molecules                                    | * Breast cancer  
* Rheumatoid arthritis                           |
| Minocycline                  | Microglia, T cells and neurons       | * Inhibits NF-κB translocation to the nucleus  
* Inhibits NFAT1                             | Acne vulgaris                                     |
| Paroxetine                   | Microglia                            | P2X4R antagonist                                                                    | Depression                                       |
| Propentofylline (Aventis Pharma)| Microglia, astrocytes and neurons    | * Inhibits cAMP and cGMP phosphodiesterases  
* Adenosine reuptake inhibitor                   | Ischaemic stroke                                  |
| Resolvin D1 (Resolvex Pharmaceuticals) | Microglia and neurons | * Attenuates pro-inflammatory cytokine release  
* Inhibits TRPV1  
* Reverses NMDAR subunit phosphorylation       | NA                                              |
| Resolvin E1 (Resolvex Pharmaceuticals) | Microglia and neurons | * Attenuates pro-inflammatory cytokine release  
* Inhibits TRPV1  
* Attenuates glutamate release  
* Reverses NMDAR subunit phosphorylation       | NA                                              |
| Rifampin                     | Microglia                            | Disrupts TLR4 signalling by binding to MD2                                          | Tuberculosis                                      |
| (+)-naloxone                 | Microglia                            | Disrupts TLR4 signalling by binding to MD2                                          | NA                                              |
| (+)-naltrexone               | Microglia                            | Disrupts TLR4 signalling by binding to MD2                                          | NA                                              |

A2A, adenosine receptor 2A; A2B, adenosine receptor 2B; cAMP, cyclic AMP; cGMP, cyclic GMP; EAAT2, excitatory amino acid transporter 2; MAPK, mitogen-activated protein kinase; MD2, myeloid differentiation protein 2; NA, not applicable (no current clinical application); NFAT1, nuclear factor of activated T cells 1; NF-κB, nuclear factor-κB; NMDAR, ionotropic glutamate receptor; P2X R, P2X purinoceptor 4; PKA, protein kinase A; PKC, protein kinase C; TLR4, Toll-like receptor 4; TRPV1, transient receptor potential cation channel subfamily V member 1
Current Goals:
Determine how the balance between algesic and analgesic mediators can be restored following tissue/nerve injury.

Determine how rodents and humans differ in the inflammatory response and pain. PPF study for PHN ....

Use human glia (and neurons) to investigate changes induced by painful conditions.

Develop ways to image glial cell activation.

Kimelberg and Nedergaard (2010)
The Inflammatory Reflex – Kevin Tracey

Homeostasis and inflammatory responses are regulated using the cholinergic anti-inflammatory pathway.

Brief vagal stimulation provides tonic inhibition of inflammation by augmenting CAP activity.
Ongoing trial in human RA, Crohn’s disease. Showed efficacy in models of Crohn’s, UC, pancreatitis, sepsis, myocardial infarction. Outcomes are lower inflammatory markers, TNF, IIs, HMGB1.
Injury activates microglia.

Primary afferents release chemokines that increase expression of P2X4R in microglia.

P2X4+ microglia signal to DH pain neurons through release of BDNF.

BDNF/trkB signaling disinhibits* L1 neurons by suppressing activity of the K⁺/Cl⁻ transporter KCC2 causing changes in GABA/glycine signaling.

The change in P2X4R+ state of microglia and the BDNF/trkB/ KCC2 disinhibition result in mechanical allodynia, hyperalgesia and spontaneous pain.

* Reduction/loss of inhibitory transmission

Bacteria activate sensory neurons that modulate pain and inflammation. (2013) Nature. Chiu et al.,

Do neurons behave as ‘immune sensors’, independent of glial and immune cells?

Inflammation-induced afferent activation independent of TLR2 and Myd88 signaling (knockout mice used).

Alternative activation pathways:
FPR1 – a GPCR pattern recognition receptor that binds bacterial formyl peptide.

ADAM10 – metalloprotease that binds staph α toxin → Ca2+ fluxes in neurons.

Inflammation-induced afferent activation independent of TLR2 and Myd88 signaling (knockout mice used).
Activation states of glia in response to injury.

1. Glial reaction: changes of glial markers and morphology
2. Regulation of receptors, channels, and transporters in glia
3. Phosphorylation of MAP kinase signaling pathways in glia
4. Production of cytokines, chemokines, growth factors in glia
   - Release of glial mediators
   - Interaction with neurons: central and peripheral sensitization
   - Persistent pain

CD11b (OX-42), IBA1, GFAP
The temporal and spatial processes involving immune and glial cells in neuropathic pain.

**Pain behaviours**
- Development of allodynia and hyperalgesia
- Maintenance of allodynia and hyperalgesia

**Time after peripheral nerve injury**
- **First 24 hours**
  - Peripheral nerve:
    - Complement activation (Li et al., 2007)
    - Mast cell degranulation (Zuo et al., 2003)
    - Peak of neutrophil infiltration (Perkins et al., 2000)
    - Schwann cells become activated and initiate Wallerian degeneration (Campana, 2007)
  - Dorsal root ganglion:
    - Activation of SGCs begins (Xie et al., 2009)
  - Spinal cord:
    - Activation of microglia begins (Tanga et al., 2004)
  - Brain:
    - Activation of microglia in the rostral ventromedial medulla (RVM) (Wei et al., 2008)

- **3 days**
  - Peripheral nerve:
    - Peak of hematogenous macrophage infiltration (Zuo et al., 2003), which has a key role in Wallerian degeneration
    - Infiltration of T lymphocytes and macrophages continues
  - Dorsal root ganglion:
    - Continued activation of SGCs
  - Spinal cord:
    - Activation of astrocytes begins (Colburn et al., 1999, Tanga et al., 2004, Romero-Sandoval et al., 2008)
  - Brain:
    - Activation of microglia in hypothalamus and periaqueductal gray (Takeda et al., 2009)

- **1-2 weeks**
  - Peripheral nerve:
    - Peak of T lymphocyte infiltration (Zuo et al., 2003)
    - Infiltration of neutrophils, macrophages (Hu et al., 2007), T lymphocytes (Hu et al., 2002)
  - Dorsal root ganglion:
    - Peak of SGC activation (Xie et al., 2009)
    - Infiltration of neutrophils, macrophages (Hu et al., 2007), T lymphocytes (Hu et al., 2002)
  - Spinal cord:
    - T cell infiltration peaks (Cao & Deleo, 2008)
    - Complement activation (Griffin et al., 2007)
    - Microglial activation peaks (Jin et al., 2003, Mika et al., 2009)
  - Brain:
    - Activation of astrocytes in the periaqueductal gray (Wei et al., 2008, Mor et al., 2010)

- **3 or more weeks**
  - Peripheral nerve:
    - Peak of T lymphocyte infiltration at 3 weeks, continues for up to 6 weeks (Moalem et al., 2004)
    - Wallerian degeneration and nerve regeneration
  - Dorsal root ganglion:
    - SGC activation continues for up to 2 months (Lee et al., 1998, Zhou et al., 1999)
  - Spinal cord:
    - Microglial activation continues for at least 3 months (Coyle, 1998)
    - Astrocytic activation continues for at least 3 months (Coyle, 1998, Deumens et al., 2009)
  - Brain:
    - Disappearance of activated microglia in the RVM (Wei et al., 2008)
Another microglia response phenotype mediated by chemokine signaling implicated in chronic pain:

- P2X4R - identified as critical element underlying neuropathic (but not inflammatory) pain.
- The development of mechanical allodynia correlates with an increase in P2X4R in *microglia*.
- Activation of P2X4R in microglia essential for neuropathic pain: blocking its function reversed pain behaviors and transfer of P2X4+ glia caused pain.
- Conversion to P2X4R+ state mediated by CCL2, CCL21, IFNγ, fibronectin, PAR2 from mast cells.

L. Sorkin lab – behavioral pain phenotype is linked to C5a; if block C5a receptor get less pain.

![Graph showing 50% withdrawal threshold over time for saline and C5aR antag with error bars.](image)

The GD$_2$ ganglioside is enriched in plasma membranes of neuroblastoma tumor cells and to a lesser extent peripheral nerves.

GD$_2$ antibodies – lyse neuroblastoma cells via complement-dependent cytotoxicity. But, treatment causes an morphine-resistant visceral pain and skin allodynia (mech, not temp).

Found a mutated GD$_2$ antibody with a point mutation that limits complement activation, and yet is effective in destroying target cells, does not cause pain.