Role of microglia in neuropathic pain, postoperative pain, and morphine tolerance

Yeong-Ray Wen1,2,3, Ping-Heng Tan1,4, Jen-Kun Cheng1,5,6,7, Yen-Chin Liu1,8, and Ru-Rong Ji1

1Sensory Plasticity Laboratory, Pain Research Center, Department of Anesthesiology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA
2Department of Anesthesiology, China Medical University Hospital, Taichung, Taiwan
3School of Medicine, China Medical University, Taichung, Taiwan
4Department of Anesthesia and Critical Care and Department of Biomedical Engineering, E-DA Hospital/I-Shou University, Kaohsiung, Taiwan
5Department of Anesthesiology, Mackay Memorial Hospital, Taipei, Taiwan
6Mackay Medicine, Nursing and Management College, Taipei, Taiwan
7Department of Anesthesiology, School of Medicine, Taipei Medical University, Taipei, Taiwan
8Department of Anesthesiology, College of Medicine, National Cheng Kung University, Tainan City, Taiwan

Abstract

Management of chronic pain such as nerve injury-induced neuropathic pain associated with diabetic neuropathy, viral infection, and cancer is a real clinical challenge. Major surgeries such as breast and thoracic surgery, leg amputation, and coronary artery bypass surgery also lead to chronic pain in 10–50% of individuals after acute postoperative pain, in part due to surgery-induced nerve injury. Current treatments mainly focus on blocking neurotransmission in the pain pathway and have only resulted in limited success. Ironically, chronic opioid exposure may lead to paradoxical pain. Development of effective therapeutic strategies requires a better understanding of cellular mechanisms underlying the pathogenesis of neuropathic pain. An important progress in pain research points to important role of microglial cells in the development of chronic pain. Spinal cord microglia are strongly activated after nerve injury, surgical incision, and chronic opioid exposure. Increasing evidence suggests that under all these conditions the activated microglia not only exhibit increased expression of microglial markers CD11b and Iba1 but also display elevated phosphorylation of p38 MAP kinase. Inhibition of spinal cord p38 has been shown to attenuate neuropathic pain and postoperative pain, as well as morphine-induced antinociceptive tolerance. Activation of p38 in spinal microglia results in increased synthesis and release of the neurotrophin BDNF and the proinflammatory cytokines IL-1β, IL-6, and TNF-α. These microglia-released mediators can powerfully modulate spinal cord synaptic transmission, leading to increased excitability of dorsal horn neurons, i.e., central sensitization, in part via suppressing inhibitory synaptic transmission. We review the studies that support the pronociceptive role of microglia in conditions of neuropathic pain, post-surgical pain, and opioid

Corresponding author: Correspondence should be addressed to Ru-Rong Ji, Department of Anesthesiology, Brigham and Women’s Hospital, 75 Francis Street, MRB 611, Boston, MA 02115, USA, Tel: (617) 732-8852; Fax: (617) 730-2801
rrji@zeus.bwh.harvard.edu.

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tolerance. Some of these studies have been accomplished by four Taiwanese anesthesiologists who are also co-authors of this review during their training at Harvard Medical School. We conclude that targeting microglial signalling may lead to more effective treatments for devastating chronic pain after diabetic neuropathy, viral infection, cancer, and major surgeries in part via improving the analgesic efficacy of opioids.

Keywords
Central sensitization; neuronal-glial interactions; proinflammatory cytokines; p38 MAP kinase; spinal cord

Microglia activation and neuropathic pain

Microglial cells originate from bone marrow-derived monocytes migrating to the central nervous system (CNS) during perinatal time and account for 5–12% of total cells in the CNS. In normal conditions, microglia are ramified and were thought to be “quiescent”. However, microglia in the non-injured conditions are not really quite, because they can actively sense their environment with their ramified processes.1 After peripheral nerve injury, microglia in the spinal cord become activated showing dramatic changes in morphology (from ramified to amoeboid) and robust increases in the expression of microglial markers such as CD11b and Iba1 (Fig. 1).2 Proliferation of microglia in the spinal cord after nerve injury is also a feature of microglia activation. In the normal conditions, glial cell proliferation is rarely detected. However, robust microglial proliferation occurs under several neuropathic pain conditions after sciatic nerve constriction, partial sciatic nerve ligation, or spared nerve injury (SNI),2–3 in which two of the three terminal branches of the sciatic nerve are ligated leaving the third brand sural nerve intact.4 Notably, nerve injury-induced cell proliferation in the spinal cord is largely restricted to microglial cells, although proliferation of other cell types such as astrocytes was also reported.5 The specific role of microglial proliferation for the control of neuropathic pain has not been clearly demonstrated. But more microglia could result in increased production of pain mediators from microglia.

While nerve injury-induced morphological changes of microglia are very striking, biochemical changes after nerve injury are more important for microglia to induce pain. Nerve injury results in a dramatic up-regulation of the ATP receptor P2X46 and the chemokine receptor CX3CR1 in spinal cord microglia.7–8 Spinal blockade of P2X4 and CX3CR1 signaling attenuates nerve injury-induced neuropathic pain.6,8 The chemokine receptor CCR2 and the Toll-like receptor-4 (TLR4) also contribute to neuropathic pain sensitization via microglial activation,9–10 although CCR2 and TLR4 localization in microglia has not been clearly demonstrated.

Studies from many laboratories in the world have demonstrated that nerve injury causes phosphorylation of p38 mitogen-activated protein kinase (MAPK) in spinal cord microglia.11–12 Phospho-p38 (p-p38) levels are low in the spinal cord of non-injured rats. Spinal nerve ligation induces a substantial increase in p-p38 levels in the injured side of the spinal cord, which is accompanied by an increase in p38 activity.11 Strikingly, p38 is primarily if not exclusively activated in spinal cells expressing the microglial markers CD11b/OX-42 and Iba1.13–14 In contrast, p-p38 is barely detected in NeuN-expressing neurons, although low-level of p-p38 may be seen occasionally. We confirmed microglia activation of p38 in the spared nerve injury model.15 p38 activation in spinal microglia was also reported after ventral root lesion16 and spinal cord injury.17 Although p38 activation peaks in the first week of nerve injury, the activation is still maintained even 3 weeks later.18
Thus, either intrathecal pre-treatment of p38 inhibitor (e.g., SB203580 and FR167653) or intrathecal post-treatment of p38 inhibitor, at early and late time of nerve injury, can effectively reduce nerve injury-mechanical allodynia, a cardinal feature of neuropathic pain. Consistently, minocycline, a non-selective microglial inhibitor attenuates neuropathic pain by inhibiting p38. Since minocycline only inhibits neuropathic pain in the early phase, it may not inhibit p38 activation in the late-phase.

What is causing the activation of p38 and microglia in the spinal cord? We have shown that matrix metalloproteinase-9 (MMP-9) can cause microglial activation via neuronal-glial interaction. Spinal nerve ligation elicits a rapid increase in MMP-9 protein and activity in DRG neurons. Intrathecal administration of MMP-9 induces persistent mechanical allodynia for many days. Intrathecal MMP-9 also induces a drastic activation of spinal microglia, as revealed by increased p38 phosphorylation and OX-42 expression in the spinal cord. A critical issue to study MMP-9 function is how to persistently suppress MMP-9 expression in the DRG. Tan and coauthors developed an RNA interference strategy to target gene expression in the pain system, using a cationic polymer, polyethyleneimine (PEI) to form a ‘proton sponge’ due to its buffering capacity, which enables PEI to buffer endosomes and induce their rupture to release small interfering RNA (siRNA) into the cytoplasm. We employed this siRNA strategy to target MMP-9 in the DRGs after nerve injury. Intrathecal injections of MMP-9 specific siRNA (2 x 5 µg) in rats effectively suppressed spinal nerve ligation-induced MMP-9 up-regulation by >70% in the DRG without affecting MMP-2 levels. Importantly, this siRNA treatment also suppressed microglia activation in the spinal cord and delayed the development of mechanical allodynia. We found that Cy3-labeled siRNA was heavily taken up by many DRG cells 3 hours after intrathecal injection. These results suggest that siRNA knockdown is an effective way to study gene functions in neuropathic pain. An association of MMP-9 with microglia activation of p38 is further validated by the finding that intrathecal p38 inhibitor can block MMP-9-induced neuropathic pain symptom, mechanical allodynia.

MMP-9, as well as ATP and chemokines (e.g., CCL2 and fractalkine (FKN)/CX3CL1) could be released from DRG neurons by nerve injury-induced discharge, causing the activation of microglia in the spinal cord (Fig. 2). It is generally believed that nerve injury-induced spontaneous discharge in the axons and cell bodies of DRG neurons can drive neuropathic pain. Indeed, blocking neural activity in the sciatic nerve by the local anesthetic bupivacaine can prevent nerve injury-induced spinal microglia activation of p38 in the spared nerve injury model. In contrast, blocking C-fiber activity in the sciatic nerve with an ultrapotent capsaicin analogue resiniferatoxin fails to inhibit p38 activation in this model. Thus, activity from large myelinated A-fibers is also important for microglia activation after nerve injury.

Microglia activation and postoperative pain

Growing evidence has indicated that post-surgical pain, traditionally regarded as acute pain and resolved spontaneously, could become chronic and persistent under similar processes. For example, groin hernia repair, breast and thoracic surgery, leg amputation, thoracotomy and coronary artery bypass surgery result in chronic pain in 10–50% individuals after acute post-surgical pain, in part due to surgery-induced nerve injury. In light of various types of surgeries in human, an optimal animal model is essential for investigating the mechanisms and treatments of postoperative pain. The most widely-used surgical pain model in rodents was developed by Brennan, et al. In this incisional pain model, a longitudinal incision (1 cm) was made in the plantar surface deep to muscle layers in a hind limb. Behaviorally, hypersensitivity to mechanical touch and radiant heat were shown to develop immediately after surgery and last for 2–3 days. Many studies demonstrated that this model is compatible
to human incisional pain in terms of behavioural, pharmacological, and molecular changes.26–28 Other surgical models have also been developed since then to mimic different conditions of human surgeries, such as back incision,29 hindlimb incision,30 and gastrocnemius incision as models of incisional pain, a thoracotomy model31 to study surgeries in nerve-rich tissues, a laparotomy model32 to mimic abdominal surgical consequences, and a skin/muscle incision and retraction (SMIR) model33 to explore potentially persistent pain following an operation at somatic tissues.

In our previous study,28 in the plantar incision model, we found that a simple brief incision at the paw induced a marked up-regulation of p38 phosphorylation in the spinal dorsal horn, starting within 1 hour, reaching peak at 1 day, and declining after 3–5 days. The time course of p38 activation was compatible with that of early pain progression after operation. Except very few neurons expressing p-p38 within the first 1 hour, we observed that activated p38 is exclusively expressed in microglia. However, change of microglia surface marker (e.g., CD11b/OX-42) after incision was found 2–3 days later, with a remarkable increase from day 3 to 7 after incision.28 The function of the delayed microglial reaction remains to be investigated.

Involvement of p38 MAPK in postoperative pain development is further confirmed by pharmacological inhibition of p38 via intrathecal route. The p38 inhibitor FR167653 produces a potent anti-inflammatory action by inhibiting the production of interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α). We found that intrathecal FR167653 prevented incision-induced mechanical allodynia and also reduced p-p38 levels in the spinal dorsal horn, but only had a mild effect on reducing thermal hyperalgesia.28 These results support the hypothesis that p38 activation in spinal microglial cells plays a critical role in the development and maintenance of postoperative mechanical hypersensitivity. This study also suggests that targeting p38 in microglia may offer a novel way of preventing persistent postoperative pain by inhibiting microglia-driven “central sensitization”, i.e. hyperactivity in spinal cord dorsal horn neurons, a critical mechanism underlying the development of persistent pain.34 In addition, Eisenach and collaborator also showed that cyclooxygenase-1 (COX-1) is dramatically up-regulated in spinal microglia after incision and intrathecal administration of a COX-1 inhibitor can attenuate post-incisional pain for several days.35 It is attempting to postulate that p38 activation in microglia induces COX-1 expression to drive incisional pain, although p38 can regulate many other targets (Fig. 2).

Microglia activation and morphine tolerance

Opioids are the primary treatment for chronic pain. Medical practice has shifted over the past decades, and long-term use of opioids is common. However, long-term administration of opioids produces negative health consequences, such as increased risk of abuse and addiction. Prolonged administration of opioids is also associated with the development of antinociceptive tolerance, wherein higher doses of the drug are required over time to elicit the same degree of analgesia. Numerous animal studies have demonstrated that sustained exposure to systemic or spinal opioids, including morphine, DAMGO, fentanyl, or heroin produces paradoxical pain, characterized as heat hyperalgesia and mechanical allodynia. Opioid-induced hyperalgesia was also found in chronic pain patients.36–37 Chronic morphine exposure results in a strong upregulation of the microglia markers CD11b and Iba1, as well as the ATP receptors P2X4 and P2X7 in spinal microglia.38–39 Intrathecal injections of antisense oligodeoxynucleotides against P2X4 or P2X7 antagonist prevent the development of morphine tolerance and microglial reaction.38–39 In particular, chronic morphine induces p38 activation in spinal microglia, and intrathecal treatment of p38 inhibitor or minocycline prevents the development of morphine tolerance.40–41
Mechanisms of microglia-evoked pain

Figure 2 illustrates how microglia activation causes pain hypersensitivity after nerve injury, surgical procedures, and chronic opioid exposure. Phosphorylation of p38 in microglia via activation of P2X4 receptor was shown to increase the synthesis and release of the neurotrophin BDNF, and BDNF could enhance neuropathic pain via suppressing inhibitory synaptic transmission in the spinal cord. Phosphorylation of p38 in microglia also results in increased synthesis of the proinflammatory cytokines IL-1β, IL-6, and TNF-α in part through the activation of the transcription factor NF-κB. Lipopolysaccharide (LPS), a potent microglia activator and also a TLR4 ligand, has been shown to induce IL-1β release via p38 activation in spinal microglia.

Accumulating evidence indicates a critical role of IL-1β, IL-6, and TNF-α in inducing hyperactivity of dorsal horn neurons, i.e. central sensitization, leading to pain hypersensitivity. Intrathecal administration of IL-1β, IL-6, and TNF-α induce robust heat hyperalgesia and mechanical allodynia. Conversely, spinal blockade of these cytokines has been shown to attenuate inflammatory pain, neuropathic pain, and morphine tolerance. Intrathecal administration of IL-1β induces a substantial increase in Cox-2 mRNA levels in the spinal cord. Perfusion of spinal cord slices IL-1β, IL-6, and TNF-α also activate the transcription factor CREB (cAMP response element-binding protein), which is critical for the transcription of pronociceptive genes such as neurokinin-1 and Cox-2 and long-term neuronal plasticity in dorsal horn neurons. In particular, we found that these proinflammatory cytokines also have non-transcriptional role in pain control. They can powerfully regulate synaptic transmission via enhancing excitatory synaptic transmission and suppressing inhibitory synaptic transmission.

Our patch clamp recordings in isolated spinal cord slices have revealed the following findings. First, IL-1β and TNF-α increase spontaneous excitatory postsynaptic currents (sEPSCs) in dorsal horn neurons and enhance AMPA- or NMDA-induced currents. Second, IL-1β and IL-6 decrease spontaneous inhibitory postsynaptic currents (sIPSCs) in dorsal horn neurons and suppress GABA- or glycine-induced currents. Similar findings on IL-1β’s actions were also reported in cultures dorsal horn neurons, and TNF-α causes disinhibition in GABAergic neurons in spinal cord slices. In addition to direct effects on synaptic transmission, TNF-α could further activate astrocytes via c-Jun N-terminal kinase (JNK) to produce monocyte chemoattactant protein-1 (MCP-1/CCL2), an important chemokine for central sensitization, whereas IL-1β can activate spinal microglia via p38 phosphorylation. Of note morphine metabolite morphine-3-glucuronide has been shown to facilitate pain via TLR4 activation and IL-1β release; and conversely, intrathecal injection of IL-1β antagonist and TLR4 inhibitor can potentiate morphine analgesia.

Conclusions and future directions

Chronic pain is an increasing burden for the society, affecting 20% of the population worldwide. Current treatments that focus mostly on targeting neuronal excitability and transmission are not satisfying. The emerging role of microglia in pain control brought great excitement to the pain research field. We have discussed the pronociceptive role of microglia in neuropathic pain, postoperative pain, and opioid tolerance. It is important to point out that some of these studies have been accomplished by four Taiwanese anesthesiologists as co-authors of this review during their training at Harvard Medical School. Apparently, microglia regulate chronic pain and opioid tolerance via neuronal-glial interactions (Fig. 2). First, primary sensory neurons exhibit hyperactivity after nerve injury, surgical procedures, and chronic opioid treatment and release potential microglial activators such as ATP, MMP-9, and the chemokines (e.g., FKN and MCP-1). Second, p38 activation in microglia leads to the production of pain mediators such as neurotrophin and cytokines to
modulate synaptic transmission and enhance pain. Thus, targeting microglial signaling via inhibiting the actions of chemokines (FKN, CCL2), ATP receptors (P2X4, P2X7), MMP-9, p38 MAPK, or proinflammatory cytokines (IL-1β, IL-6, and TNF-α) may lead to novel therapies for chronic pain.

Finally, we have to point out that apart from microglia, other types of glial cells such as astrocytes are also important for inflammatory and neuropathic pain.59–60 Our work in progress has shown that astrocytes can produce tissue plasminogen activator (tPA), a protease in the spinal cord to facilitate morphine tolerance (Liu YC and Ji RR, unpublished observation). Satellite glial cells share similar molecular features as astrocytes but are localized in the dorsal root ganglion (DRG) in the peripheral nervous system. Activation of satellite cells in the DRG after morphine treatment could antagonize morphine analgesia via releasing IL-1β (Liu YC and Ji RR, unpublished observation). It remains to be investigated how different types of glial cells control pain sensitivity under various injury and treatment conditions.

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References


Figure 1. Microglial reaction in the spinal dorsal horn of rats after nerve injury and paw incision. (A) OX-42 immunofluorescence (dark field) in the dorsal horn one day after spinal nerve ligation. The lesion side shows marked microglial activation in comparison with the contralateral side. (B) Immunohistochemical staining (bright field) of Iba-1 in the dorsal horn one day after plantar incision. The reactive microglia in the injured side display a dense and amoeboid appearance in contrast to the ramified morphology of microglia in the contralateral side. Scar bar: 100 μm.
Figure 2.
Schematic illustration of microglia-evoked pain. Nerve injury, surgical procedures, and chronic opioid exposure result in activation of microglial cells in the spinal cord. This activation could be initiated by the release of ATP, matrix metalloprotease-9 (MMP-9), and the chemokine fractalkine (FKN/CX3CL1), leading to the phosphorylation of p38 MAPK in microglia. Activation of p38 induces the synthesis and release of several pain mediators including the proinflammatory cytokines (IL-1β, IL-6, and TNF-α) and neurotrophin (BDNF) from microglia. These glia-produced pain mediators can initiate and maintain postoperative pain, neuropathic pain, and antinociceptive tolerance of opioids, via inducing hyperexcitability of nociceptive neurons in the spinal cord dorsal horn.