



## Review Article

## Microglia: A Promising Target for Treating Neuropathic and Postoperative Pain, and Morphine Tolerance

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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, partly 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 might lead to paradoxical pain. Development of effective therapeutic strategies requires a better understanding of cellular mechanisms underlying the pathogenesis of neuropathic pain. Progress in pain research points to an 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 mitogen-activated protein kinase. Inhibition of spinal cord p38 has been shown to attenuate neuropathic 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 brain-derived neurotrophic factor and the proinflammatory cytokines interleukin-1 $\beta$ , interleukin-6, and tumor necrosis factor- $\alpha$ . These microglia-released mediators can powerfully modulate spinal cord synaptic transmission, leading to increased excitability of dorsal horn neurons, that is, central sensitization, partly via suppressing inhibitory synaptic transmission. Here, we review studies that support the pronociceptive role of microglia in conditions of neuropathic and postoperative pain and opioid tolerance. We conclude that targeting microglial signaling might lead to more effective treatments for devastating chronic pain after diabetic neuropathy, viral infection, cancer, and major surgeries, partly via improving the analgesic efficacy of opioids.

**Key Words:** central sensitization, neuronal–glial interactions, p38 mitogen-activated protein kinase, proinflammatory cytokines, spinal cord

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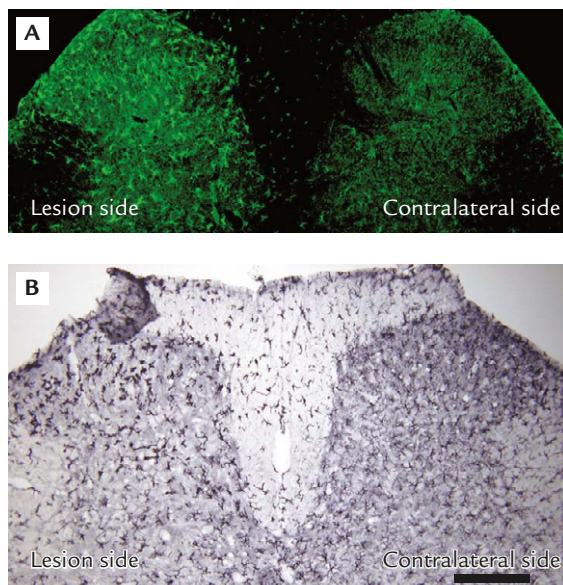
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## Microglia Activation and Neuropathic Pain

Microglial cells originate from bone-marrow-derived monocytes migrating to the central nervous system during the perinatal period, and they account for 5–12% of the total cells in the central nervous system. Under normal conditions, microglia are ramified and thought to be quiescent. However, microglia under the non-injured conditions are not really quiescent, because they can actively sense their environment with their ramified processes.<sup>1</sup> After peripheral nerve injury, microglia in the spinal cord become activated and show dramatic changes in morphology (from ramified to ameboid) and robust increases in the expression of microglial markers such as CD11b and Iba1 (Figure 1).<sup>2</sup> Proliferation of microglia in the spinal cord after nerve injury is also a feature of microglial activation. Under normal conditions, glial cell proliferation is rarely detected.



**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 1 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 1 day after plantar incision. The reactive microglia in the injured side displayed a dense and ameboid appearance in contrast to the ramified morphology of microglia in the contralateral side. Scar bar = 100  $\mu$ m.

However, robust microglial proliferation occurs under several neuropathic pain conditions after sciatic nerve constriction, partial sciatic nerve ligation, or spared nerve injury (SNI),<sup>2,3</sup> in which two of the three terminal branches of the sciatic nerve are ligated, leaving the third branch, the sural nerve, intact.<sup>4</sup> 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, has also been reported.<sup>5</sup> The specific role of microglial proliferation in the control of neuropathic pain has not been clearly demonstrated. However, more microglia could result in increased production of pain mediators.

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

Studies from many laboratories worldwide have demonstrated that nerve injury causes phosphorylation of p38 mitogen-activated protein kinase (MAPK) in spinal cord microglia.<sup>11,12</sup> 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.<sup>11</sup> Strikingly, p38 is primarily if not exclusively activated in spinal cells expressing the microglial markers.

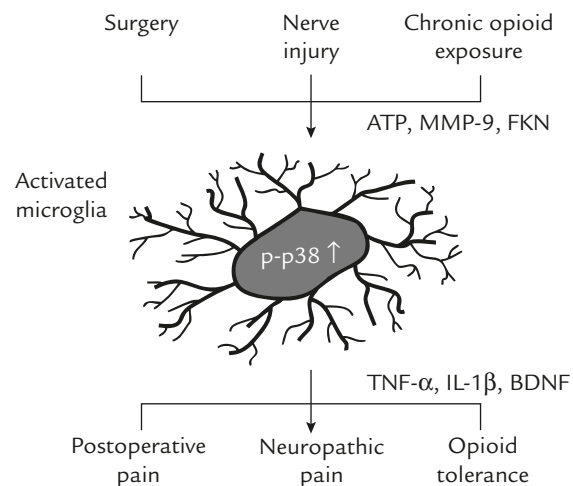
CD11b/OX-42 and Iba1.<sup>13,14</sup> By contrast, p-p38 is barely detected in Neuronal-nuclei-expressing neurons, although low levels of p-p38 might be seen occasionally. We have confirmed microglial activation of p38 in the SNI model.<sup>15</sup> p38 activation in spinal microglia has also been reported

after ventral root lesion<sup>16</sup> and spinal cord injury.<sup>17</sup> Although p38 activation peaks in the first week of nerve injury, activation is still maintained even 3 weeks later.<sup>18</sup> Thus, either intrathecal pretreatment of p38 inhibitor (e.g. SB203580 or FR167653) or intrathecal post-treatment with p38 inhibitor, at early and late stages of nerve injury, can effectively reduce nerve injury mechanical allodynia, a cardinal feature of neuropathic pain.<sup>13–15</sup> Consistently, minocycline, a non-selective microglial inhibitor attenuates neuropathic pain by inhibiting p38.<sup>17,19</sup> Minocycline only inhibits neuropathic pain in the early phase; therefore, it might not inhibit p38 activation in the late phase.

What causes activation of p38 and microglia in the spinal cord? We have shown that matrix metalloproteinase (MMP)-9 can cause microglial activation via neuronal–glial interaction. Spinal nerve ligation elicits a rapid increase in MMP-9 protein and activity in dorsal root ganglion (DRG) neurons.<sup>20</sup> Intrathecal administration of MMP-9 induces persistent mechanical allodynia for many days.<sup>20</sup> Intrathecal MMP-9 also induces drastic activation of spinal microglia, as revealed by increased p38 phosphorylation and OX-42 expression in the spinal cord.<sup>20</sup> A critical issue to study MMP-9 function is how to suppress MMP-9 expression persistently in the DRG. Tan and coauthors<sup>21</sup> have developed an RNA interference strategy to target gene expression in the pain system, using a cationic polymer, polyethyleneimine, to form a “proton sponge” by utilizing its buffering capacity, which enables polyethyleneimine to buffer endosomes and induce their rupture to release small interfering RNA (siRNA) into the cytoplasm.<sup>21,22</sup> We have used this siRNA strategy to target MMP-9 in the DRG after nerve injury. Intrathecal injections of MMP-9-specific siRNA ( $2 \times 5 \mu\text{g}$ ) in rats effectively suppressed spinal-nerve-ligation-induced MMP-9 upregulation by  $>70\%$  in the DRG without affecting MMP-2 levels.<sup>20</sup> Importantly, this siRNA treatment also suppressed microglia activation in the spinal cord and delayed the development of mechanical allodynia.<sup>20</sup> We found that Cy3-labeled

siRNA was heavily taken up by many DRG cells 3 hours after intrathecal injection.<sup>22</sup> 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 has been validated by the finding that intrathecal p38 inhibitor can block the MMP-9-induced neuropathic pain symptom, mechanical allodynia.<sup>20</sup>

MMP-9, as well as ATP and chemokines [e.g. CC chemokine ligand (CCL) 2 and fractalkine (FKN)/CX3CL1] are released from DRG neurons by nerve-injury-induced discharge, which causes activation of microglia in the spinal cord (Figure 2). It is generally believed that nerve-injury-induced spontaneous discharge in the axons and cell bodies of DRG neurons can drive neuropathic pain.<sup>23</sup> Indeed, blocking neural activity in



**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, MMP-9, and the chemokine FKN, leading to the phosphorylation of p38 mitogen-activated protein kinase in microglia. Activation of p38 induces the synthesis and release of several pain mediators including the proinflammatory cytokines (IL-1 $\beta$  and TNF- $\alpha$ ) and 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. MMP = matrix metalloproteinase-9; FKN = fractalkine; p-p38 = phospho-p38; IL-1 $\beta$  = interleukin-1 $\beta$ ; TNF- $\alpha$  = tumor necrosis factor- $\alpha$ ; BDNF = brain-derived neurotrophic factor.

the sciatic nerve by the local anesthetic bupivacaine can prevent nerve-injury-induced spinal microglia activation of p38 in the SNI model.<sup>15</sup> By contrast, blocking C-fiber activity in the sciatic nerve with an ultrapotent capsaicin analog, resiniferatoxin, fails to inhibit p38 activation in this model.<sup>24</sup> Thus, activity from large myelinated A-fibers is also important for microglial activation after nerve injury.

### Microglial Activation and Postoperative Pain

Growing evidence has indicated that postoperative pain, traditionally regarded as acute spontaneously resolving pain, 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 postoperative pain, partly due to surgery-induced nerve injury.<sup>25</sup> In light of various types of surgery 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.<sup>26</sup> In this incisional pain model, a longitudinal incision (1 cm) was made in the plantar surface deep to the muscle layers in a hind limb.<sup>26</sup> Behaviorally, hypersensitivity to mechanical touch and radiant heat was shown to develop immediately after surgery and lasted for 2–3 days. Many studies have demonstrated that this model is compatible with human incisional pain in terms of behavioral, pharmacological, and molecular changes.<sup>26–28</sup> Other surgical models have also been developed since then to mimic different conditions of human surgery, such as: back incision,<sup>29</sup> hindlimb incision,<sup>30</sup> and gastrocnemius incision as models of incisional pain; a thoracotomy model<sup>31</sup> to study surgery in nerve-rich tissues; a laparotomy model<sup>32</sup> to mimic abdominal surgical consequences; and a skin/muscle incision and retraction model<sup>33</sup> to explore potentially

persistent pain following surgery of the somatic tissues.

In our previous study,<sup>28</sup> we found in the plantar incision model that a simple brief incision at the paw induced marked upregulation of p38 phosphorylation in the spinal dorsal horn, starting within 1 hour, reaching a 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 for a few neurons expressing p-p38 within the first hour, we observed that activated p38 was exclusively expressed in microglia. However, changes in microglia surface markers (e.g. CD11b/OX-42) after incision was found 2–3 days later, with a marked increase from Day 3 to Day 7 after incision.<sup>28</sup> The function of the delayed microglial reaction remains to be investigated.

Involvement of p38 MAPK in postoperative pain development has been confirmed by pharmacological inhibition of p38 via the intrathecal route. The p38 inhibitor FR167653 produces a potent anti-inflammatory action by inhibiting the production of interleukin (IL)-1 $\beta$  and tumor necrosis factor (TNF)- $\alpha$ . We have found that intrathecal FR167653 prevents incision-induced mechanical allodynia, and also reduces p-p38 levels in the spinal dorsal horn, but only has a mild effect on reducing thermal hyperalgesia.<sup>28</sup> These results support the hypothesis that p38 activation in spinal microglial cells plays a crucial role in the development and maintenance of postoperative mechanical hypersensitivity. This study has also suggested that targeting p38 in microglia offers a novel way of preventing persistent postoperative pain by inhibiting microglia-driven “central sensitization”, that is, hyperactivity in spinal cord dorsal horn neurons, a crucial mechanism underlying the development of persistent pain.<sup>34</sup> In addition, Eisenach and collaborators have also shown that cyclooxygenase (COX)-1 is dramatically upregulated in spinal microglia after incision, and intrathecal administration of a COX-1 inhibitor can attenuate post-incisional pain for several days.<sup>35</sup> It is tempting to postulate that p38 activation in microglia induces COX-1

expression to drive incisional pain, although p38 can regulate many other targets (Figure 2).

### Microglia Activation and Morphine Tolerance

Opioids are the primary treatment for acute and cancer pain.<sup>36,37</sup> Medical practice has shifted over recent decades, and opioid use in chronic pain has become 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 (D-Ala<sup>2</sup>, N-MePhe<sup>4</sup>, Gly-ol]-enkephalin), fentanyl, or heroin produces paradoxical pain, characterized as heat hyperalgesia and mechanical allodynia. Opioid-induced hyperalgesia is also found in chronic pain patients.<sup>38,39</sup> Chronic morphine exposure results in a strong upregulation of the microglial markers CD11b and Iba1, as well as the ATP receptors P2X<sub>4</sub> and P2X<sub>7</sub> in spinal microglia.<sup>40,41</sup> Intrathecal injections of antisense oligodeoxynucleotides against P2X<sub>4</sub> or P2X<sub>7</sub> antagonists prevent development of morphine tolerance and microglial reaction.<sup>40,41</sup> In particular, chronic morphine induces p38 activation in spinal microglia, and intrathecal treatment with p38 inhibitor or minocycline prevents the development of morphine tolerance.<sup>42,43</sup>

### Mechanisms of Microglia-evoked Pain

Figure 2 illustrates how microglial activation causes pain hypersensitivity after nerve injury, surgical procedures, and chronic opioid exposure. Phosphorylation of p38 in microglia via activation of P2X<sub>4</sub> receptor increases the synthesis and release of the neurotrophin brain-derived

neurotrophic factor (BDNF), and BDNF could enhance neuropathic pain via suppressing inhibitory synaptic transmission in the spinal cord.<sup>44</sup> Phosphorylation of p38 in microglia also results in increased synthesis of the proinflammatory cytokines IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , partly through activation of the transcription factor nuclear factor- $\kappa$ B.<sup>11,45</sup> Lipopolysaccharide, a potent microglia activator and also a TLR4 ligand, induces IL-1 $\beta$  release via p38 activation in spinal microglia.<sup>46</sup>

Accumulating evidence indicates a crucial role for IL-1 $\beta$ , IL-6 and TNF- $\alpha$  in inducing hyperactivity of dorsal horn neurons, that is, central sensitization, leading to pain hypersensitivity.<sup>47,48</sup> Intrathecal administration of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  induces robust heat hyperalgesia and mechanical allodynia.<sup>48,49</sup> Conversely, spinal blockade of these cytokines has been shown to attenuate inflammatory and neuropathic pain, and morphine tolerance.<sup>50-54</sup> Intrathecal administration of IL-1 $\beta$  induces a substantial increase in COX-2 mRNA levels in the spinal cord.<sup>55</sup> Perfusion of spinal cord slices with IL-1 $\beta$ , IL-6 or TNF- $\alpha$  also activates the transcription factor cAMP response element-binding protein,<sup>48</sup> which is crucial for transcription of pro-nociceptive genes such as neurokinin-1 and COX-2, as well as long-term neuronal plasticity in dorsal horn neurons.<sup>34</sup> In particular, we have found that these proinflammatory cytokines also have a non-transcriptional role in pain control. They can powerfully regulate synaptic transmission via enhancing excitatory synaptic transmission and suppressing inhibitory synaptic transmission.<sup>48</sup> Our patch clamp recordings in isolated spinal cord slices have revealed the following. First, IL-1 $\beta$  and TNF- $\alpha$  increase spontaneous excitatory postsynaptic currents in dorsal horn neurons, and enhance 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid- or N-methyl D-aspartic acid-induced currents. Second, IL-1 $\beta$  and IL-6 decrease spontaneous inhibitory postsynaptic currents in dorsal horn neurons and suppress  $\gamma$ -aminobutyric acid- or glycine-induced currents.<sup>48,49,53</sup> Similar findings for the actions of IL-1 $\beta$  have also been reported in cultured dorsal horn neurons,<sup>56</sup> and TNF- $\alpha$

causes disinhibition in GABAergic neurons in spinal cord slices.<sup>57</sup> In addition to direct effects on synaptic transmission, TNF- $\alpha$  can further activate astrocytes via c-Jun N-terminal kinase, to produce monocyte chemoattractant protein-1 (CCL2), an important chemokine for central sensitization,<sup>58</sup> whereas IL-1 $\beta$  can activate spinal microglia via p38 phosphorylation.<sup>24,59</sup> Of note, morphine metabolite morphine-3-glucuronide facilitates pain via TLR4 activation and IL-1 $\beta$  release; conversely, intrathecal injection of IL-1 $\beta$  antagonist and TLR4 inhibitor can potentiate morphine analgesia.<sup>60</sup>

## Conclusions and Future Directions

Chronic pain is an increasing burden for society, affecting 20% of the population worldwide. Current treatments that focus mostly on targeting neuronal excitability and transmission are unsatisfactory. 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, who are also co-authors of this review, during their training at Harvard Medical School. Apparently, microglia regulate chronic pain and opioid tolerance via neuronal-glia interactions (Figure 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 monocyte chemoattractant protein-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 (e.g. FKN or CCL2), ATP receptors (e.g. P2X4 or P2X7), MMP-9, p38 MAPK, and/or proinflammatory cytokines (e.g. IL-1 $\beta$ , IL-6 or TNF- $\alpha$ ) might 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.<sup>61,62</sup> Our work in progress has shown that astrocytes can produce tissue plasminogen activator, a protease in the spinal cord to facilitate morphine tolerance (unpublished observations). Satellite glial cells share similar molecular features as astrocytes, but are localized in the DRG in the peripheral nervous system. Activation of satellite cells in the DRG after morphine treatment could antagonize morphine analgesia via release of IL-1 $\beta$  (unpublished observations). It remains to be investigated how different types of glial cells control pain sensitivity under various injury and treatment conditions.

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