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Microglia in Pain: Detrimental and protective roles in pathogenesis and resolution of pain

Gang Chen[1], **Yu-Qiu Zhang**[2], **Yawar J Qadri**[3], **Charles N Serhan**[4], and **Ru-Rong Ji**[3,5,6]

[1]Key Laboratory of Neuroregeneration of Jiangsu and Ministry of Education, Co-Innovation Center of Neuroregeneration, Nantong University, Nantong, Jiangsu 226001, China

[2]Institute of Neurobiology, Institutes of Brain Science and State Key Laboratory of Medical Neurobiology, Collaborative Innovation Center for Brain Science, Fudan University, Shanghai 200032, China

[3]Department of Anesthesiology, Duke University Medical Center, Durham, North Carolina, 27710, USA

[4]Center for Experimental Therapeutics and Reperfusion Injury, Hale Transformative Medicine, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

[5]Department of Neurobiology, Duke University Medical Center, Durham, North Carolina, 27710, USA

[6]Lead Author

Abstract

The previous decade has seen a rapid increase in microglial studies on pain, with a unique focus on microgliosis in the spinal cord after nerve injury and neuropathic pain. Numerous signaling molecules are altered in microglia and contribute to the pathogenesis of pain. Here we discuss how microglial signaling regulates spinal cord synaptic plasticity in acute and chronic pain conditions with different degrees and variations of microgliosis. We highlight that microglial mediators such as pro- and anti-inflammatory cytokines are powerful neuromodulators that regulate synaptic transmission and pain via neuron-glial interactions. We also reveal an emerging role of microglia in the resolution of pain, in part via specialized pro-resolving mediators including resolvins, protectins and maresins. We also discuss a possible role of microglia in chronic itch.

Keywords

Inflammation; itch; microglia; neuroinflammation; nerve injury; neuropathic pain; specialized pro-resolving mediators; spinal cord

Corresponding authors: Ru-Rong Ji, ru-rong.ji@duke.edu.

Microglia are macrophage-like cells in the central nervous system (CNS) which regulate homeostasis in the brain and spinal cord. During development, microglia originate from erythromyeloid progenitors in the yolk sac and develop in the forming CNS (Crotti and Ransohoff, 2016). Increasing evidence suggests that microglia are a heterogeneous population throughout the CNS and play an active role in maintaining normal physiological conditions, as they sense the cellular environment with their ramified processes and undergo rapid morphological changes in response to mediators such as ATP (Davalos et al., 2005; Hanisch and Kettenmann, 2007). During development microglia dynamically interact with synapses to modify their structures and functions in the healthy brain. For example, microglial processes engulf synapses and induce synaptic pruning during critical developmental stages, which involves the activation of the classic complement cascade (e.g. neuronal C1q and microglial C3 receptors) (Stevens et al., 2007). Microglia are long-lived cells, with a median lifetime of well over 15 months, and half of all microglia survive for the entirety of a mouse's lifespan (Fuger et al., 2017).

Microglia are emerging as key regulators of brain diseases. These include neurodegenerative diseases like such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, stroke, neuropsychiatric diseases for instance depression, and anxiety, and neurodevelopmental diseases such as autism (Crotti and Ransohoff, 2016; Hanisch and Kettenmann, 2007; Mosser et al., 2017; Salter and Stevens, 2017; Zhan et al., 2014). Microglia contribute to the pathogenesis of brain diseases via regulation of neuroinflammation, a localized inflammation of the peripheral and central nervous system (Heppner et al., 2015; Ji et al., 2014; Ransohoff, 2016). A PubMed query reveals hundreds to thousands of publications demonstrating the role of microglia in each of these diseases (Figure 1A).

Microglia and Pain

Notably, there are over sixteen hundred publications on microglia and pain, showing a rapid increase in last 10 years (Figure 1B). While the majority of studies on microglia and brain diseases focus on how microglia effect synaptic pruning and neurodegeneration, pain researchers searched for neuromodulators produced by microglia that can rapidly modulate synaptic plasticity, a driving force for the pathogenesis of pain after tissue and nerve injury (Luo et al., 2014; Woolf and Salter, 2000). The injury-induced synaptic plasticity in the spinal cord and brain pain circuits is also termed central sensitization, which sustains chronic pain and causes widespread pain beyond the initial injury site (Ji, 2018; Woolf, 1983). Another striking difference between pain and other neurological diseases is the rapid onset of pain: microglia regulate neuronal and synaptic activities to change pain behavior within minutes to tens of minutes following treatment with microglial activators and inhibitors (Berta et al., 2014; Tsuda et al., 2003b).

Peripheral nerve injury not only results in neuropathic pain, which is characterized by mechanical allodynia, a pain evoked by normally innocuous stimulation such as light touch, but also causes remarkable microgliosis in the spinal cord. Gliosis is a nonspecific reactive change of glial cells in response to injuries and insults and often involves the proliferation or hypertrophy of glial cells. Microgliosis manifests as profound morphological changes,

where microglia transition from ramified to amoeboid shapes with enlarged cell bodies and shortened processes. This was first reported in 1975 when Gilmore showed proliferation of non-neuronal cells (also called “neuro-glia) in spinal cords of irradiated, immature rats following transection of the sciatic nerve (Gilmore, 1975). Gilmore and Skinner further reported that sciatic nerve injury in adult rats also resulted in a proliferation of non-neuronal cells in the spinal cord using Nissl and haematoxylin and eosin staining (Gilmore and Skinner, 1979). In 1993, Eriksson and his coworkers demonstrated remarkable microgliosis in the pain-modulating spinal cord and brain stem regions after nerve injury using immunostaining of OX-42, an antibody that recognizes complement receptor CR3 (CD11b) (Eriksson et al., 1993), with others reproducing a profound microglial proliferation, evident within 2 days after nerve injury (Beggs and Salter, 2007; Echeverry et al., 2008; Suter et al., 2007).

Insights into the specific role of microglia in pain were revealed in 2003 when three separate groups showed microglial involvement in neuropathic pain after peripheral nerve injury (Jin et al., 2003; Raghavendra et al., 2003; Tsuda et al., 2003b). Jin et al. reported that spinal nerve ligation evokes phosphorylation of p38 MAP kinase (P-p38, an active form of p38) only in CD11b (OX-42)-expressing spinal microglia but not in GFAP-expressing astrocytes or NeuN-expressing neurons, and furthermore, intrathecal injection of a p38 inhibitor reduced mechanical allodynia, a cardinal feature of neuropathic pain after nerve injury (Jin et al., 2003). Raghavendra et al. showed that minocycline, a non-specific microglial inhibitor, inhibited mechanical hyperalgesia and allodynia in the early phase but not the late phase after spinal nerve transection. This inhibition in the early phase of nerve injury was associated with inhibition of microglial activation in the spinal cord (Raghavendra et al., 2003). Tsuda et al. demonstrated that spinal injection of ATP-activated microglia was sufficient to evoke rapid mechanical allodynia within one hour of treatment. Furthermore, they found (1) nerve injury results in upregulation of the ATP receptor subtype P2X4 exclusively in spinal cord microglia and (2) spinal inhibition of P2X4 attenuated mechanical allodynia (Tsuda et al., 2003b). In 2004, Tsuda et al. also confirmed the role of p38 in spinal microglia for the development of mechanical allodynia after nerve injury (Tsuda et al., 2004). In 2005, Coull et al. demonstrated that spinal microglia produce the brain-derived neurotrophic factor (BDNF) to drive neuropathic pain (Coull et al., 2005).

A major issue in microglia research is the lack of selective pharmacological tools. Grace and collaborators developed Gq- and Gi-coupled DREADDs (Designer Receptor Exclusively Activated by a Designer Drug) for selective activation or inhibition of microglia. DREADDs under a CD68 promoter were intrathecally transfected via an AAV9 vector to target microglia/macrophages. Activation of microglia with Gq (stimulatory) DREADD, which is stimulated by its clozapine-N-oxide (CNO) ligand, is sufficient to induce mechanical allodynia via IL-1 secretion, as this allodynia can be abolished by intrathecal interleukin-1 receptor antagonist. In contrast, nerve injury-induced allodynia is attenuated by intrathecal activation of Gi (inhibitory) DREADD (Grace et al., 2016; Grace et al., 2018). This chemogenetic approach further supports a critical role of spinal microglia in establishing mechanical allodynia.

There are a number of excellent reviews that focus on the role microglia in neuropathic pain (Clark and Malcangio, 2012; Inoue and Tsuda, 2018; McMahon and Malcangio, 2009; Salter and Stevens, 2017; Tsuda et al., 2005). In this review, we offer a more comprehensive view on microglia in various pain states with different degrees of microgliosis. In addition to neuropathic pain with nerve injury, we discuss microgliosis and microglial signaling in inflammatory pain, cancer pain, and drug-induced pain after chemotherapy and chronic opioid exposure. In particular, we present a balanced view of microglia, in which microglia are both detrimental and protective in their effects, contributing to both the pathogenesis and resolution of pain.

Microglia Regulate Neuropathic Pain after Peripheral Nerve Injury

Microglia in the spinal cord horn are strongly activated after peripheral nerve injury (Guan et al., 2016; Tsuda et al., 2005). Spinal microglial activation after nerve injury requires neuronal activity (Wen et al., 2007; Xie et al., 2009). While C-fiber activation is sufficient to elicit spinal microglial activation (Gruber-Schoffnegger et al., 2013; Hathway et al., 2009), activation of large A-fibers is also important to maintain microglial activation (Suter et al., 2009).

Microglial Activators Are Initially Released from Primary Sensory Neurons

Multiple signaling molecules released from damaged primary afferents play a crucial role in the induction and development of spinal microgliosis. Guan et al. show that peripheral nerve injury induces a rapid increase in the expression of colony stimulating factor 1 (CSF1) in injured DRG neurons (Guan et al., 2016; Okubo et al., 2016). The CSF1 released from damaged primary afferents acts on spinal microglia to induce microgliosis and pain behaviors. Spinal injection of CSF1 inhibitor (Okubo et al., 2016) or Cre-mediated deletion of *Csf1* in DRG neurons (Guan et al., 2016) both attenuate nerve injury induced microgliosis and mechanical allodynia. Furthermore, intrathecal injection of CSF1 in naïve mice induced mechanical hypersensitivity and microgliosis (Guan et al., 2016).

Sensory neurons also release several chemokines after nerve injury to activate microglia. CCL2 is strongly upregulated in DRG neurons by nerve injury and contribute to neuropathic pain via CCR2 receptor (White et al., 2005). Although CCR2 was implicated in microglial activation in neuropathic pain (Abbadie et al., 2003; Thacker et al., 2009; Zhang et al., 2007), microglial expression of CCR2 in spinal dorsal horn has not been clearly demonstrated in the spinal cord dorsal horn. Instead, CCR2 expression was reported in DRG and spinal cord neurons (Gao et al., 2009; White et al., 2005). It is well documented that CXCL1, released from DRG neurons, induces microgliosis and mechanical allodynia via CX3CR1 (Clark and Malcangio, 2012). Furthermore, liberation of CXCL1 from the DRG cell surface requires cathepsin S (Clark et al., 2009). Additionally, CCL21 is rapidly induced in a small population of DRG neurons and enhances microglial activation and neuropathic pain via CCR7 receptor (Biber et al., 2011).

Neuronal proteases also play an important role in microglia activation. Nerve injury induces a rapid and transient upregulation of metalloproteinase-9 (MMP-9) expression in injured DRG neurons, which contributes to the induction but not maintenance of neuropathic pain

(Ji et al., 2009). Intrathecal MMP-9 administration produces neuropathic pain symptoms and microgliosis through IL-1β cleavage. Conversely, MMP-9 inhibition or knockdown reduces microgliosis and mechanical allodynia (Kawasaki et al., 2008a). Caspase 6, a cysteine-aspartic acid protease, is specifically expressed by axonal terminals in the spinal cord and released to the cerebrospinal fluid by neuronal activation (Berta et al., 2014). Nerve injury causes a profound upregulation of caspase-6 mRNA in injured DRG neurons, which contributes to microglial activation and neuropathic pain (Berta et al., 2017; Berta et al., 2016). Extracellular application of caspase-6 triggers a substance TNF release from microglia, leading to TNF-dependent pain following intrathecal injection (Berta et al., 2014).

Following peripheral nerve injury, Neuregulin-1 is also released from primary afferents to activate ErbB receptors on spinal microglia. Intrathecal injection of neuregulin-1 induced microgliosis and development of pain hypersensitivity via phosphorylation of ERK1/2 and Akt in microglia (Calvo et al., 2010).

Up-regulation of Microglial Signaling Molecules after Nerve Injury

Once microglia are activated by the activators released after nerve injury, various microglial signaling molecules are up-regulated in the spinal cord dorsal horn, including cell surface receptors, as well as intracellular and secreted signaling molecules (Table 1).

Purinergic Receptors.—ATP is a prominent activator of microglia. There are three subtypes of purinergic receptors, the adenosine P1 receptors, the P2X ion channel receptors, and the G protein-coupled P2Y receptors. Nerve injury up-regulation of P2X4R exclusively occurs in spinal cord microglia. Spinal blockade of P2X4R signaling reduces nerve injury-induced mechanical allodynia, a cardinal feature of neuropathic pain. Furthermore, nerve injury-induced allodynia is abolished in *P2×4r* knockout mice and intrathecal injection of P2X4R-stimulated microglia can cause allodynia in normal rats (Tsuda et al., 2003a). These results indicate that P2X4R on spinal microglia is a key receptor for establishing mechanical allodynia. Neuronal CCL21 upregulates microglial P2X4 via activation of the CCR7 receptor (Biber et al., 2011). Nerve injury also induces up-regulation of P2X7R in spinal cord microglia (Kobayashi et al., 2011). P2X7R inhibitors reduce mechanical allodynia in neuropathic pain models by intraperitoneal or intrathecal injection. In addition, *P2×7r* knockout mice have significantly reduced pain hypersensitivities after peripheral nerve injury. Interestingly, P2X7R and P2X4R can form a functional interaction through their physical association in macrophages (Boumechache et al., 2009; Perez-Flores et al., 2015). It will be interesting to study whether this interaction also occurs in spinal microglia and contributes to neuropathic pain. In addition to P2X subtypes, peripheral nerve injury also induced robust increases of P2Y6R, P2Y12R, P2Y13R, and P2Y14R mRNA expression in spinal microglia. The functional blockade of all these P2Y receptors all reduced pain hypersensitivity, indicating that the microglial P2Y receptors and ATP signaling may also play an active role in neuropathic pain (Kobayashi et al., 2012).

Toll-Like Receptor (TLRs).—The TLR family includes 14 subtypes and plays a crucial role in the innate immune response (Liu et al., 2012). The role of TLR4 in nerve injury-

induced neuropathic pain has been extensively studied. *Tlr4* expression in spinal cord rapidly increased four hours after L5 spinal nerve transection (Tanga et al., 2004). *Tlr4*-deficient mice show attenuated mechanical allodynia and thermal hyperalgesia after nerve injury (Tanga et al., 2005). Similarly, intrathecal antisense knockdown of spinal TLR4 in wild-type mice inhibits nerve injury-induced microglial activation and cytokine expression and neuropathic pain (Tanga et al., 2005). Furthermore, intrathecal injection of various TLR4 antagonists reverse nerve injury induced mechanical allodynia and thermal hyperalgesia both in mice and rats (Nicotra et al., 2011). These results suggest that TLR4 plays an important role in the initiation and maintenance of spinal microglia activation and neuropathic pain. However, astrocytic expression of TLR4 was shown in spinal dorsal horn under some pathological conditions (Li et al., 2014; Liu et al., 2016). Future studies of conditional deletions of *Tlr4* in microglia and astrocytes are required. *Tlr2* knockout mice also exhibit reduced neuropathic pain and spinal microglia activation in some nerve injury models (Kim et al., 2007).

Chemokine Receptors.—There receptors are widely expressed in the nervous system and are involved in neuropathic pain. In the spinal cord, the chemokine receptor CX3CR1 is exclusively expressed in microglia and is upregulated after nerve injury (Zhuang et al., 2007). In *Cx3cr1* knockout mice, partial sciatic nerve ligation injury-induced spinal microglial activation is suppressed and mechanical allodynia does not occur (Clark et al., 2011; Staniland et al., 2010b).

MAP Kinases (MAPKs).—After nerve injury, phosphorylation of p38 MAPK is increased and highly restricted to spinal microglia (Jin et al., 2003; Tsuda et al., 2004). Pharmacological inhibition of p38 MAPK activity or knockdown of the p38α isoform via intrathecal route suppressed the development of mechanical allodynia in various models of neuropathic pain (Jin et al., 2003; Luo et al., 2017; Tsuda et al., 2004). Besides p38 MAPK, phosphorylation of extracellular signal-regulated kinases 1/2 and 5 (ERK1/2 and ERK5), was also found in spinal microglia after nerve injury and both ERK1/2 and ERK5 regulate neuropathic pain (Calvo et al., 2011; Obata et al., 2007; Zhuang et al., 2005). Inhibition of the ERK1/2 pathway and Rho-associated coiled-coil-containing protein kinase (ROCK) reduced microgliosis and pain hypersensitivity after peripheral nerve injury (Calvo et al., 2011; Tatsumi et al., 2015) (Figure 2A).

Src-Family Kinases.—Src family has five members in the CNS Src, Lyn, Lck, Yes, and Fyn (Salter and Kalia, 2004). Nerve injury elicits a striking increase in Src phosphorylation in spinal microglia, and activated Src contributes to the development of neuropathic pain (Katsura et al., 2006). ERK is downstream of Src activation and mediates Src-induced chemokine release. Intrathecal injection of Src inhibitor attenuates mechanical allodynia by suppressing ERK activity in spinal microglia (Katsura et al., 2006). Lyn is also upregulated in spinal microglia after nerve injury and activates ERK, leading to increased P2X4R expression in microglia after nerve injury. Moreover, *Lyn* deletion reduces tactile allodynia after nerve injury (Tsuda et al., 2008).

Proteases: Peripheral nerve injury induces cathepsin S (CatS) in spinal microglia, and spinal administration of CatS inhibitor is antihyperalgesic and antiallodynic in neuropathic rats (Clark et al., 2007). Interestingly, CatS is a secreted protease, which induces neuropathic pain via cleavage and liberation of CXCL1 (fractalkine), leading to further p38 activation in spinal microglia (Clark et al., 2007; Clark et al., 2009).

Transcription Factors.—Interferon regulatory factor-8 (IRF8) expression is exclusively upregulated in spinal microglia after nerve injury (Masuda et al., 2012). IRF8 activates many genes that are involved in microglial responses and promote neuropathic pain (Masuda et al., 2015; Masuda et al., 2012). Knockdown of IRF8 expression in the spinal cord caused a recovery of tactile allodynia. Furthermore, nerve injury induced neuropathic pain is attenuated in IRF8 knockout mice (Masuda et al., 2012). In addition, IRF8 regulates the expression of IRF1 and IRF5 in microglia. While the IRF8-IRF1 cascade regulates IL-1β expression (Masuda et al., 2015), the IRF8-IRF5 cascade regulates P2X4R expression (Masuda et al., 2014).

Compliment Cascade: Peripheral nerve injury also upregulates complement components C1q, C3, and C4 in spinal microglia. Notably, C1q and C4 are among the most strongly regulated genes by nerve injury. Furthermore, nerve injury results in upregulations of the terminal complement component C5 and the C5a receptor (C5aR) in spinal microglia, and neuropathic pain is attenuated by deletion of C5 or by C5aR antagonist (Griffin et al., 2007).

Distinct Responses of Microglia: Microgliosis and Microglia Activation

A key aspect of the microglial response to nerve trauma is microgliosis, including microglial morphological changes and proliferation (Figure 2A). Following peripheral nerve injury, in addition to morphological changes, spinal microglia begin to proliferate within 2 to 3 days and reach maximal levels in 4 to 7 days (Echeverry et al., 2008). Normally, microgliosis after nerve injury is a transient and self-limited event, and microglia return to normal levels within a few weeks. Nerve injury induced microgliosis is concomitant with the development of pain hypersensitivity. Accordingly, blocking microgliosis attenuates pain behaviors (Gu et al., 2016; Guan et al., 2016; Sorge et al., 2015). Microglial proliferation is partially controlled by CX3CR1 and P2Y12, and inhibiting the proliferation of microglia effectively alleviated neuropathic pain during the development phase (Gu et al., 2016; Peng et al., 2016). It is important to point out that both spinal microgliosis and neuropathic pain development after nerve injury are age dependent and do not occur during the first several weeks of life due to the masking effect of anti-inflammatory cytokines such as IL-10 (McKelvey et al., 2015).

Resident vs. Migrating Microglia.—Although early studies using radiation revealed a contribution of bone marrow-derived monocytes to microglial proliferation in the spinal cord after nerve injury (Zhang et al., 2007), more recent work using mouse genetic models, such as CCR2(RFP/+):CX3CR1(GFP/+) mice demonstrates that microglial proliferation in the spinal cord after nerve injury resulted from resident microglia but not from infiltrating monocytes (Gu et al., 2016). Further, using parabiosis and mice with genetically labelled microglia, Tashima et al. demonstrated little contribution of circulating bone marrow-derived

cells to spinal microglial proliferation after peripheral nerve injury (Tashima et al., 2016). Interestingly, spinal microglia can be ablated selectively using intrathecal injection of Mac-1-saporin, a microglia selective immunotoxin. And they will repopulate rapidly within several days, but monocyte CCR2 signaling is not involved in this microglia repopulation (Yao et al., 2016).

Microgliosis vs. Microglia Activation.—Notably, microgliosis is not always correlated with pain states, and neuropathic pain can be reduced without changes of microgliosis (Ji et al., 2013; Tsuda et al., 2003b). For example, in *P2×4*-deficient mice nerve injury induced spinal microgliosis is normal, but pain behaviors are inhibited (Ulmann et al., 2008). Thus, microglia could be activated in the absence of microgliosis. It has been widely accepted that activated microglia release bioactive mediators to facilitate pain signaling at both spinal and supraspinal levels to contribute to neuropathic pain (Figure 2B). Importantly, microglial activation via p38 phosphorylation is a common pathway after the activation of cell surface receptors on microglia, such as CX3CR1, P2X4, P2X7, P2Y12, and TLR4 (Clark et al., 2010; Ji et al., 2013; Ji and Suter, 2007; Kobayashi et al., 2008; Trang et al., 2009). Upon activation, p38 MAPK increases the synthesis and release of various microglial mediators, such as TNF-α, IL-1β, IL-6, BDNF, PGE2 and facilitates the development of neuropathic pain (Ji et al., 2013).

Do Microglia Play a Role in Other Pathological Pain Conditions?

While nerve injury induces prominent microgliosis in the spinal cord, microgliosis after other painful insults such as inflammation, arthritis, cancer, and drug treatments (e.g., chemotherapy or chronic opioid exposure) is largely variable in part due to the degree of nerve injury in these insults (Ji et al., 2013). We discuss distinct roles of microglial signaling in these pathological pain conditions, which can be dissociated from microgliosis.

Acute Inflammatory Pain

Microgliosis takes days to manifest after nerve injury. Thus, it was initially believed that microglia exclusively regulate chronic pain such as neuropathic pain with marked microgliosis but has no role in acute inflammatory pain conditions in which microgliosis is not as evident and robust. Emerging evidence suggests that microglial signaling regulates acute inflammatory pain, especially the formalin-induced 2nd phase spontaneous pain, which manifests behaviorally as licking and flinching of the animal's affected hindpaw. Since this spontaneous pain only lasts for less than 60 min, there is no obvious microgliosis in the spinal cord during this acute pain phase (Berta et al., 2014). Notably, hind paw injection of formalin also causes nerve injury, neuropathic pain, and spinal microgliosis in the late-phase after 1–3 days (Fu et al., 1999). Several lines of pharmacological and genetic evidence point to a role of spinal microglia in regulating formalin-induced 2nd phase spontaneous pain. First, intrathecal minocycline produced a dose-dependent reduction of spontaneous pain in rats and mice (Chen et al., 2018; Hua et al., 2005). Second, activation of p38 in spinal microglia is necessary for spontaneous pain in rats and mice, and this activation regulates microglial synthesis and release of PGE2 and TNF (Ji and Suter, 2007; Svensson et al., 2003a; Svensson et al., 2003b; Taves et al., 2016). Of note, formalin-induced

spontaneous pain involves TNF signaling and is substantially attenuated in mice lacking receptors for TNF (TNFR1 or TNFR2) (Zhang et al., 2011). Third, Caspase-6 drives acute inflammatory pain via microglial TNF secretion (Berta et al., 2014). Formalin spontaneous pain is abolished in mice lacking *Casp6* or after spinal inhibition of Caspase-6 (Berta et al., 2014). These data suggest that microglia regulate formalin-induced spontaneous pain via transcription-independent mechanisms. Thus, it is not surprising that this pain is not affected in mice lacking the transcription factor IRF5 (PMID 24818655).

Chronic Inflammatory Pain

The role of microglia in chronic inflammatory pain is controversial. *P2×4* knockout mice exhibit unaltered inflammatory pain following intraplantar CFA injection (Tsuda et al., 2009). However, *Cx3cr1* knockout mice exhibit reduced inflammatory pain following intraplantar zymosan injection (Staniland et al., 2010a). Both intraplantar or intra-articular injections of CFA induce persistent inflammatory pain, but microgliosis is more robust after joint CFA inoculation, suggesting that deep tissue injury is more effective in evoking microglial changes (Ji et al., 2013; Sun et al., 2007). Spinal microgliosis is also evident after collagen-induced poly-arthritis (Nieto et al., 2015). Upregulation and activation of microglial CX3CR1 is essential for arthritic pain (Sun et al., 2007).

Cancer Pain

In rodent models of cancer pain, there is a large variation in spinal microglial reaction, likely due to differences in species/strains and origins of cancer cells. In a rat model of bone cancer pain induced by inoculation of breast cancer cells (Walker 256) into the tibia, there is marked microgliosis in the spinal cord and inhibition of microglia suppresses the bone cancer-induced pain (Yang et al., 2015). Notably, spinal microglia maintain the advanced-phase of bone cancer pain, where a delayed but persistent spinal microglial activation occurs 14 to 21 days after tumor inoculation. Spinal inhibition of microglial signaling by minocycline and inhibitors of P2X7 and p38 effectively reduced established allodynia and hyperalgesia on day 14, whereas pretreatment with minocycline did not block the development of bone cancer pain. Moreover, the P2X7R/p38/IL-18 pathway was shown to maintain bone cancer pain (Yang et al., 2015). In addition, P2X4, P2Y12, and TLR4 also play an activate role in microglial activation and bone cancer pain (Liu et al., 2017; Mao-Ying et al., 2012; Meng et al., 2017). Microglia produce proinflammatory cytokines such as TNF, IL-1b, and IL-18 to elicit central sensitization and facilitate cancer pain (Liu et al., 2017; Mao-Ying et al., 2012; Yang et al., 2015). Strong microgliosis is also found in the trigeminal spinal subnucleus caudalis following inoculation of squamous cell carcinoma cells into the tongue of rats, and this oral cancer pain condition requires P2Y12R (Tamagawa et al., 2016). Microgliosis is less remarkable in murine models of bone cancer (Hald et al., 2009; Honore et al., 2000). Bone cancer pain is not affected in mice by *P2×7* deletion or P2X7 antagonists (Hansen et al., 2011). However, follow-up studies from different groups demonstrated moderate microgliosis in the spinal cord using the same bone cancer model (Pevida et al., 2014; Vit et al., 2006). Therefore, microglia may play different roles in different rodent models of cancer pain.

Neuropathic pain after spinal cord injury (SCI)

Compared with nerve injury, the temporal and spatial activation of microglia caused by SCI is much more rapid and extensive. Temporally, the expression of CD11b robustly increases from 2 hours to 180 days post-injury. Spatially, persistent microglial reaction manifests in the entire spinal axis from cervical to lumbar regions (Gwak et al., 2012), as well as supraspinal areas such as the posterolateral nucleus of thalamus, the hippocampus and the anterior cingulate cortex (Galan-Arriero et al., 2014). Intrathecal minocycline not only reduced microgliosis and p38 activation but also suppressed spontaneous and evoked activity in spinal cord neurons and mechanical hypersensitivity in SCI animals (Hains and Waxman, 2006).

Chemotherapy-induced peripheral neuropathy (CIPN)

CIPN is a dose-limiting neurotoxic effect. Although increased spinal microglial activity was reported in rats treated with high-dose of paclitaxel (Peters et al., 2007), other reports failed to show microglial activity increase following the chemotherapy agents paclitaxel (Zheng et al., 2011), oxaliplatin, or bortezomib, despite prominent astrocyte activation in these chemotherapy models (Robinson et al., 2014). Spinal knockdown of p38-a isoform of MAP kinase using potent anti-sense oligodeoxynucleotide reduced mechanical allodynia after nerve injury but not paclitaxel treatment in mice (Luo et al., 2017). Mild microglial activation with TNF and p-p38 upregulation were observed in the spinal cord following vincristine treatment in mice (Shen et al., 2015). Hu et al. demonstrated persistent activation of microglia but not astrocytes in mice for more than 5 weeks following cisplatin-CIPN. Intrathecal injection with minocycline not only alleviated mechanical allodynia but also prevented sensory deficits and loss of epidermal nerve fibers after cisplatin treatment. In spinal microglia, cisplatin induced neuroinflammation via triggering receptor expressed on myeloid cells 2 (TREM2), and functional blockade of TREM2 signaling inhibited CIPN (Hu et al., 2018). Thus, the contribution of microglia to CIPN also depends on chemotherapeutic agents and doses.

Opioid-Induced Tolerance and Hyperalgesia

Long-term administration of opioids not only increases risk of abuse and addiction, but also produces antinociceptive tolerance and paradoxical pain (hyperalgesia) (Ferrini et al., 2013; Mao et al., 1995). Selective ablation of spinal microglia prevented the development but not maintenance of morphine tolerance (Leduc-Pessah et al., 2017). Chronic morphine exposure augmented the expression of P2X4 and P-p38 in spinal microglia, and furthermore, morphine tolerance is abrogated by spinal inhibition of p38 (Cui et al., 2006) or antisense knockdown of P2X4R (Horvath et al., 2010). Differently, Ferrini et al. found that pharmacological blockade or knock-out of P2X4R reversed chronic morphine-induced hyperalgesia, but did not affect morphine analgesic tolerance (Ferrini et al., 2013). Blockade or knockdown of spinal P2X7R attenuated morphine tolerance, microglial activation, and p-p38 and IL-18 levels (Chen et al., 2012; Zhou et al., 2010). Leduc-Pessah et al. revealed a key site (Y382–384) at P2X7R that can be targeted to reduce the development of morphine tolerance (Leduc-Pessah et al., 2017). Pharmacological blockade of TLR4 signaling attenuated the development of analgesic tolerance, hyperalgesia, and opioid withdrawal

behavior in rats. Morphine may bind directly to and activate TLR4 by molecular modeling and in vitro cellular assays (Hutchinson et al., 2010; Watkins et al., 2009). However, mice lacking functional TLR4 still developed hyperalgesia with morphine treatment, suggesting that TLR4 is not required for opioid-induced analgesic tolerance or hyperalgesia (Ferrini et al., 2013; Fukagawa et al., 2013). Moreover, chronic morphine treatment prolongs the duration of nerve injury-induced neuropathic pain, and this prolongation requires the activation of NLRP3 (NOD-like receptor protein 3) inflammasome in spinal microglia (Grace et al., 2016). Panx1 hemichannels regulate ATP release from microglia, which is required for morphine withdrawal without affecting opiate analgesia (Burma et al., 2017). Although chronic exposure causes direct activation of microglia, it is still under debate whether microglia express functional opioid receptors. Notably, loss of mu opioid receptor signaling in nociceptors, but not microglia, abrogates morphine tolerance (Corder et al., 2017).

Microglial Mediators Regulate Synaptic Plasticity and Central Sensitization in Spinal Cord Pain Circuits

Spinal Cord Pain Circuits

One of the most important questions regarding microglial control of pain is how microglial mediators regulate synaptic transmission in the pain circuits. In the past 5 years, great progress has been made in dissecting the spinal cord circuits of pain (Braz et al., 2014; Duan et al., 2018; Peirs and Seal, 2016). While earlier studies focused on lamina I projection neurons due to its critical contribution to hyperalgesia (Mantyh et al., 1997; Todd, 2010), recent progress points to important contribution of interneurons, especially lamina II neurons to pain processing. For example, somatostatin-expressing (SOM^+) interneurons are essential for mediating mechanical pain (Duan et al., 2014). Transcriptional profiling of SOM^+ neurons also reveals distinct subpopulations (Chamessian et al., 2018). These interneurons form a spinal pain circuit by connecting to TRPV1-expressing C-fibers and lamina-I projection neurons (Figure 3A) (Braz et al., 2014; Todd, 2010) and also exhibit robust neural plasticity after nerve injury (Xu et al., 2013b).

Microglia Drive Central Sensitization

Chronic pain is driven by central sensitization, a phenomenon of synaptic plasticity, and increased neuronal responsiveness in central pain circuits after painful insults (Latremoliere and Woolf, 2009). Activation of NMDA receptor (NMDAR), phosphorylation of ERK (pERK), and loss of inhibition are key events that drive central sensitization in spinal cord dorsal horn neurons and pain hypersensitivity after injury (Ji et al., 2003) (Figure 3C–D). Increasing evidence suggests that microglia drive central sensitization via microglial mediators. Microglia are a major source of cytokines including TNF and IL-1β (Hanisch, 2002).

Single-cell PCR analysis reveals that in the spinal cord dorsal horn *Tnf* mRNA is synthesized by microglia and some astrocytes but not neurons (Berta et al., 2014). TNF is a powerful neuromodulator of synaptic transmission in the spinal cord pain circuit. Notably, TNF acts on synapses at low nM concentrations, in contrast to μM concentrations of classic

neurotransmitters such as glutamate (Kawasaki et al., 2008b; Park et al., 2011). Perfusion of TNF very rapidly (i.e. within a minute) increases spontaneous excitatory postsynaptic current (sEPSC) frequency in lamina II neurons via activation of TRPV1 in pre-synaptic terminals, an effect that is abolished in *Trpv1* knockout mice (Park et al., 2011). Although TNFR1 is the predominant receptor mediating TNF's effects in glial cells and neurons, TNFR2 also contributes to TNF-induced central sensitization (Zhang et al., 2011). TNF potentiates the function of NMDA receptors (NMDARs) in lamina II neurons through pERK (Xu et al., 2010). TNF also causes delayed but sustained increase in AMPAR and NMDAR mediated and evoked currents in spinal lamina I neurons (Gruber-Schoffnegger et al., 2013).

In spinal slices, microglia release IL-1β via the activation of TLR4 and P2X7 receptors and p38 (Clark et al., 2010). In dorsal horn neurons, IL-1β increases the phosphorylation of NMDAR and ERK and enhances NMDA currents (Kawasaki et al., 2008b; Zhang et al., 2008). After nerve injury IL-1β enhances the function of presynaptic NMDARs, leading to increased glutamate release and excitatory synaptic transmission (Yan and Weng, 2013).

PGE2, a prominent inflammatory mediator could also be produced from spinal cord microglia following the activation of p38 and plays an active role in central sensitization (Kohno et al., 2008; Svensson et al., 2003b). In addition to direct actions on neurons, cytokines (e.g., TNF and IL-1β) can also act on glial cells and show slow actions in lamina I neurons via secretion of PGE2 (Gruber-Schoffnegger et al., 2013).

Disinhibition as a Common Feature of Microglial Mediators

Disinhibition— the reduction or loss of inhibitory synaptic transmission in the SDH pain circuit— has been strongly implicated in central sensitization (Latremoliere and Woolf, 2009). Disinhibition often occurs after nerve injury when GABA synthesis or potassium/chloride transporter (KCC2) expression are down-regulated (Coull et al., 2003; Moore et al., 2002). Disinhibition in dorsal horn neurons also occurs in acute pain conditions, such as capsaicin-induced hyperalgesia/allodynia, and spinal injection of glycine or GABA receptor antagonists is sufficient to induce rapid allodynia in naïve animals (Lu et al., 2013). Notably, microglial mediators such as cytokines, PGE2, and BDNF modulate inhibitory synaptic transmission in spinal dorsal horn neurons via pre-, post-, and extra-synaptic mechanisms (Ji et al., 2013) (Figure 3D).

Proinflammatory cytokines powerfully modulate inhibitory synaptic transmission at multiple sites in the spinal dorsal horn (Ji et al., 2013). At the pre-synaptic level, IL-1β and IL-6 inhibit the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in spinal pain circuit. At post-synaptic sites, IL-1β and IL-6 reduce the sIPSC amplitude. At extra-synaptic sites, GABA and glycine receptor activity (revealed by GABA and glycine induced currents) can be suppressed by IL-1β (Kawasaki et al., 2008b; Yan et al., 2015). Further, TNF rapidly inhibited spontaneous action potentials in GABAergic neurons (Zhang et al., 2010).

Nerve injury releases ATP to activate P2X4R on spinal microglia, causing BDNF release. BDNF acts on TrkB in lamina I neurons and then increases the level of intracellular Cl^- by downregulating the neuronal potassium-chloride transporter, KCC2. This change leads to potentiation of synaptic GluN2B-NMDAR currents by Fyn kinase and action potential firing

in lamina I neurons (Coull et al., 2005; Hildebrand et al., 2016), resulting in neuropathic pain. However, it is difficult to detect BDNF in spinal microglial cells, although it can be easily labeled in DRG neurons and primary afferents in the spinal cord. Nociceptor-derived BDNF regulates acute and inflammatory but not neuropathic pain (Zhao et al., 2006). Sensory neuron-derived BDNF also contributes to the transition from acute to chronic neuropathic pain (Sikandar et al., 2018). Thus, the cellular locations are still under the investigation.

In addition, PGE2 inhibits glycinergic neurotransmission in spinal cord neurons via post-synaptic GlyR3 and the cAMP/PKA pathway (Harvey et al., 2004). A recent report shows that after inflammation, microglia causes displacement or retraction of inhibitory synapses in the dorsal horn (Kambrun et al., 2018). It will be interesting to learn if this retraction of inhibitory synapses is mediated by pro-inflammatory cytokines.

Regulation of Spinal Cord Long-Term Potentiation (sLTP)

Spinal cord long-term potentiation (sLTP) is an important form of spinal cord synaptic plasticity that contributes to central sensitization and pathological pain (Luo et al., 2014; Sandkuhler, 2000, 2009). Activation of spinal microglia is both sufficient and required for the induction sLTP at C-fiber synapses with spinal lamina I neurons. TNF and IL-1β are individually sufficient and necessary for sLTP induction via redundant pathways in these neurons (Clark et al., 2015; Gruber-Schoffnegger et al., 2013). Spinal TNF modulates sLTP through both TNFR1 and TNFR2 (Liu et al., 2007; Park et al., 2011). Interestingly, caspase-6 contributes to the induction and maintenance of sLTP by triggering TNF release from microglia (Berta et al., 2014). IL-1β triggers sLTP not only in excitatory synapses (Gruber-Schoffnegger et al., 2013) but also in glycinergic synapses on GABAergic neurons in spinal cord slices, as another example of disinhibition (Chirila et al., 2014). Activation of spinal microglial CX3CR1 via CX3CL1/fractalkine is sufficient to elicit sLTP. Specifically, the activation of the CX3CR1 receptor by fractalkine induces the release of IL-1β from microglia, which modulates NMDA signaling in postsynaptic neurons, leading to the release of an eicosanoid messenger, that ultimately enhances presynaptic neurotransmitter release. In contrast to the conventional view, this form of plasticity does not require enhanced neuronal activity to trigger the events leading to synaptic facilitation in lamina I neurons (Clark et al., 2015).

Recently, Sandkuhler and coworkers proposed “gliogenic LTP” that can spread widely in nociceptive pathways (Kronschlager et al., 2016). A fundamental feature of LTP induction in the brain is the requirement for coincident pre- and post-synaptic activity, which is important to restrict LTP expression to activated synapses only (homosynaptic LTP) as well as to define the input specificity of LTP. Gliogenic sLTP can travel long distances via CSF, because this LTP can be induced by glial activation and diffusible messengers, such as D-serine and TNF. Remarkably, transfer of spinal CSF from a donor animal displaying LTP is able to induce LTP in a naïve recipient animal (Kronschlager et al., 2016). Therefore, this diffusible sLTP can affect susceptible synapses at remote sites. Collectively, gliogenic LTP, as well as other forms of diffusible and glial-mediated central sensitization may underlie

widespread pain in chronic pain patients, especially in those with overlapping chronic pain conditions (Ji et al., 2018).

Sex Dimorphism in Microglial Signaling in Pain

Clinical pain syndromes such as chronic orofacial pain or temporomandibular disorders occur more frequently in women compared to men. Although 70% of pain clinic patients are women, the majority of preclinical studies have been conducted in male rodents (Mogil, 2012). It is noteworthy that the issue of "Sex As a Biological Variable (SABV)" must be addressed in grant applications to the National Institutes of Health. Despite the estrous cycle in female rodents, females do not show fluctuations of basal mechanical and thermal pain sensitivity when tested at different times during the cycles. Recent evidence shows sex dimorphism in pain processing by immune cells especially microglial cells. Spinal TLR4 regulates inflammatory pain and neuropathic pain in male but not female mice (Sorge et al., 2011), highlighting possible male-dominant microglial signaling in SDH. Furthermore, two studies found equivalent microgliosis in the spinal dorsal horn of male and female mice in response to peripheral nerve injury, accompanied by similar levels of allodynia (Sorge et al., 2015; Taves et al., 2016). However, elimination of spinal microglia with a toxin or spinal inhibition of microglial function with minocycline, P2X4 blocker, or microglial BDNF signaling inhibits mechanical allodynia only in male but not female mice. Also, nerve injury upregulates P2X4 expression in the spinal cord of male mice (Sorge et al., 2015). Interestingly, this male-specific microglial response depends on testosterone, as minocycline did not inhibit allodynia in castrated males but reduced allodynia in testosterone-treated females. It appears that female rodents switch from microglial signaling to T-cell signaling in neuropathic pain and have more T-cells and exhibit increased T-cell marker expression after injury (Sorge et al., 2015).

We also compared nerve injury-induced microglial proliferation and microglial activation markers in both sexes. CCI induced comparable microgliosis and expression of CX3CR1 and IBA-1 in both sexes. However, nerve injury induced p-p38 in spinal microglia predominantly in male mice (Taves et al., 2016). Therefore, only some changes in microglia such as p-p38 but not IBA-1 are sex-dependent. CCI induced comparable synaptic plasticity, i.e. increases in EPSCs in SDH neurons in both sexes. Strikingly, skepinone, a selective p38 inhibitor, not only attenuated CCI-induced mechanical allodynia exclusively in males, but also suppressed CCI-induced synaptic plasticity in males (Taves et al., 2016). Consistently, spinal inhibition of P2XR or p38 signaling was shown to disrupt hyperalgesic priming, a persistent pain condition induced by IL-6 and subsequent challenge with PGE2, in male but not female mice (Paige et al., 2018). Therefore, sex-specific p38 activation and signaling appears to be confined to the spinal cord in inflammatory and neuropathic pain conditions. Notably, delayed microglial activation also contributes to bone cancer pain in female rats inoculated with breast cancer cells (Yang et al., 2015). Thus, sex-dimorphism in microglial regulation of pain depends on pain models and injury conditions (Table 2). Notably, sex dimorphism in microglial signaling was not noticed in mice lacking *Cx3cr1* or *Tmem16f* or after microglia deletion (Batti et al., 2016; Peng et al., 2016; Staniland et al., 2010a), but sex difference was not the focus of these studies the data of both sexes are often combined.

Future rigorous studies are necessary to determine the pain conditions and microglial pathways that are sex-dependent.

Villa et al. described significant differences in the transcriptome of adult male and female microglia due to perinatal exposure to sex steroids. Strikingly, microglia isolated from adult brains maintain the sex-specific features when put in culture or transplanted in the brain of the opposite sex. Furthermore, female microglia are neuroprotective by restricting focal cerebral ischemia damage (Villa et al., 2018). It is of great interest to assess whether female microglia are also protective against the development of chronic pain. The microbiome also influences prenatal and adult microglia in a sex-specific manner. Antibiotic treatment of adult mice triggered sexually biased microglial responses, revealing both acute and long-term effects of microbiota depletion (Thion et al., 2018).

It is noteworthy that astrocytes also play an important role in the pathogenesis of chronic pain (Ji et al., 2013). Persistent upregulation of astrocytic connexin-43 (Cx43) sustains mechanical allodynia after nerve injury via chemokine release (e.g., CXCL1) (Chen et al., 2014). In contrast to sex dimorphism in microglial signaling, astroglial signaling exhibits no apparent sex-differences, and intrathecal injection of Cx43 blocker and astroglial toxin effectively reduced FSP and mechanical allodynia in both sexes (Chen et al., 2018).

Protective Role of Microglia in Resolution of Neuroinflammation and Pain

Protection of Neuroinflammation in Neurological Diseases

Despite a critical role of microglia in establishing neuroinflammation and pathogenesis of neurological and neuropsychiatric diseases (Ji et al., 2014), these long-lived cells also contribute to homeostasis of the brain and spinal cord and resolution of neuroinflammation (Shemer et al., 2015). For example, microglia-related genes have been implicated in neuropsychiatric or neurological disorders, such as frontotemporal dementia (*TREM2*), Alzheimer's disease (*CD33*), and hereditary diffuse leukencephalopathy (*CSF1R)* (Erny et al., 2015). In particular, mice lacking *Cx3cr1* exhibited greater cognitive dysfunction and increased neuronal death in the chronic phase of traumatic brain injury compared to wild-type mice, despite the detrimental role of microglia in the acute phase where microglial activity correlated with motor deficits and cell death. These distinct behavioral phenotypes in the acute versus chronic phase of brain injury in *Cx3cr1*–deficient mice may be associated with different microglial phenotypes of these phases (Febinger et al., 2015).

Recent progress has demonstrated that microglial behavior can also be controlled remotely by the gut, as the microbiome can influence both prenatal and adult microglia (Thion et al., 2018). Erny et al (Erny et al., 2015) demonstrated substantial contributions of the host microbiota to microglia homeostasis, as germ-free mice exhibited global defects in microglia and impaired innate immune responses. Eradication of host microbiota altered microglia properties profoundly, and furthermore, the microglial defects could be restored partially by recolonization with a complex microbiota. Intriguingly, bacterial fermentation products such as short-chain fatty acids, which include acetic acid, propionic acid, and butyric acid, are vital for immune cell homeostasis in the colon. They may also regulate

microglia homeostasis via short-chain fatty acid receptor FFAR2 (GPR43), which is expressed on splenic Iba1+ myeloid cells but not in microglial cells (Erny et al., 2015).

Rothhammer et al. (Rothhammer et al., 2018) reported an unexpected immunoregulatory role for a ligand-activated transcription factor, the aryl hydrocarbon receptor (AHR), activated by gut-derived metabolites such as tryptophan. Elimination of microglial AHR substantially exacerbated EAE (experimental autoimmune encephalomyelitis), a mouse model of multiple sclerosis, but left immune responses outside the CNS unaltered. This finding suggests that AHR activation in microglia inhibits inflammation in the CNS. When activated in microglia, AHR serves as a transcription factor to regulate the expression of VEGF-B (down-regulation) and TGF-α (up-regulation). While VEGF-B released from microglia enhances the responsiveness of astrocytes, TGF-α dampens astrocyte responsiveness. Thus, AHR-mediated distinct regulation of VEGF-B and TGF in microglia results in the suppression of astrogliosis and neuroinflammation (Wekerle, 2018) (Figure 4).

Cannabinoid receptor type 2 (CB2) activation induces an anti-inflammatory phenotype of microglia via inducing the expression of MAP kinase phosphatase and subsequent suppression and MAP kinase phosphorylation, as an essential step for the resolution of neuropathic pain (Romero-Sandoval et al., 2009). G protein-coupled receptor kinase 2 (GRK2) is also a regulator of pain resolution, and loss of GRK2 in microglia results in a transition from acute to chronic inflammatory pain (Willemen et al., 2010). Despite recent progress in revealing microglia's protective actions in neurological disorders, their roles in resolving acute and chronic pain remain to be fully appreciated. Notably, in early life during the first several weeks, microgliosis and neuropathic pain after nerve injury are masked by the anti-inflammatory cytokine IL-10, presumably produced by microglia (McKelvey et al., 2015; Moss et al., 2007).

Specialized Pro-resolving Mediators (SPMs) in Pain Resolution

Using lipid mediator metabololipidomics, proteomics (liquid chromatography-tandem mass spectrometry [LC-MS-MS]), and cell trafficking in self-limited inflammatory exudates, the Serhan lab identified three new families of potent and pro-resolution mediators (Bannenberg et al., 2005; Hong et al., 2003; Serhan et al., 2000b; Serhan et al., 2002) (Figure 4A) coined resolvins (*resolution-phase interaction products*), protectins/neuroprotectin, and maresins (*macrophage mediators in resolving inflammation*) (Serhan et al., 2009). Each family is structurally distinct and biosynthesized from separate precursor n-3 fatty acid (FA) substrates, EPA (eicosapentaenoic acid), docosapentaenoic acid (n-3DPA), and DHA (docosahexaenoic acid) (Serhan et al., 2008). Notably, aspirin triggers their biosynthesis, and both acetaminophen and indomethacin also permit some 18-HEPE and 15-HEPE biosynthesis in contrast to the cyclooxygenase-2 (COX-2) inhibitors, which substantially reduce their formation (Serhan et al., 2000a). These findings give a new mechanism for aspirin's many well-appreciated benefits namely triggering the biosynthesis of endogenous mediators that help to terminate acute inflammation (Gilroy, 2005). In addition, lipoxins biosynthesized from arachidonic acid stop neutrophil infiltration and promote resolution as well as reduce inflammatory and neuropathic pain (Martini et al., 2016; Serhan, 2014; Svensson et al., 2007). Because each member of these families possesses potent pro-

resolving and anti-inflammatory actions (recently reviewed in Serhan, 2014, 2017) with special functions in the resolution phase, this super-family is coined “specialized pro-resolving mediators” (SPMs).

The complete stereochemistry and biosynthesis of each major SPM is established (Figure 4A). These major SPM biosynthesis routes are each confirmed with human leukocytes via matching with synthetic compounds, trapping of proposed transient epoxide-containing intermediates and label tracking of precursors and intermediates (Serhan et al., 2015) and are biosynthesized by microglial cells (Serhan et al., 2002) where they reduce cytokine actions on microglia cells. The D-series resolvins (RvDs) are biosynthesized from DHA and DPA, and the E-series resolvins (RvEs) from EPA precursor (Figure 4A). These precursors are enriched in marine oils and enter humans *via* nutrition or supplementation. NPD1 produced in neural systems is termed neuroprotectin D1 (NPD1) demonstrating potent protective actions in retina, brain, and pain (Asatryan and Bazan, 2017; Bazan et al., 2010) and is biosynthesized by microglial cells and brain (Hong et al., 2003). When biosynthesized in the immune system, this mediator is denoted PD1, reflecting its system of origin.

The stereoselective and low SPM-doses required to stop ongoing inflammation and promote resolution rely on GPCR receptors that transduce intracellular signals. To date, six separate SPM receptors are identified using library screening, labeled-ligands for specific binding (stereospecific nM K_d) and functional cellular responses (Figure 4B). Several of the resolvin and SPM receptors are present on neurons and glial cells in neural tissues (Ji et al., 2011). SPMs, except for NPD1 (Bang et al., 2018), generally do not stimulate increases in intracellular Ca^{2+} mobilization in leukocytes for signal transduction but rather activate phosphorylation demonstrated using genetically engineered mice and mass cytometry (Chiang et al., 2017; Norris et al., 2017). Each SPM interacts with specific receptors in the pico-nanomolar range with K_d in the nM range. For example, RvE1 specifically binds to Chemerin Receptor 23 (ChemR23, also known as ERV-1) (Arita et al., 2005) and Leukotriene B4 receptor 1 (BLT1) to evoke pro-resolving responses. RvE1 activation of ChemR23 enhances macrophage phagocytosis via phosphoprotein-mediated signaling (Ohira et al., 2010). The RvE1 receptor ERV/ChemR23 is expressed on DRG neurons and reduces pain signals in part via blocking the function of TRPV1 ion channels (Xu et al., 2010). RvD1 binds and activates human GPR32 and shares human and murine LXA_4 receptor (ALX/FPR2) to evoke rapid impedance changes. Transgenic mice overexpressing human ALX-FPR2 require less RvD1 to stop inflammation (Krishnamoorthy et al., 2012), RvD2 specifically binds and activates GPR18 with high affinity, regulating sepsis and bacterial clearance (Chiang et al., 2015; Chiang et al., 2017). NPD1/PD1 shows high affinity specific binding on human leukocytes and retinal epithelial cells with ^{3}H-labeled-NPD1 (Marcheselli et al., 2010) that likely represents specific receptors. Notably, GPR37 is expressed by macrophages and required for mediating NPD1-induced phagocytosis. Unlike other SPMs, NPD1 evokes slow but sustained intracellular Ca^{2+} mobilization in macrophages via GPR37. Functionally, GPR37 is required for the resolution of inflammatory pain (Bang et al., 2018). Receptors for the remaining SPMs such asMaR1 (Serhan et al., 2012) are currently subject of investigations.

Purified cell bulk analysis reveals that microglia express multiple SPM receptors, such as ChemR23 (RvE1 and RvE2 receptor, ERV1), GPR32 (RvD1 receptor, DRV1), and GPR18 (RvD2 receptor) (Thion et al., 2018). Notably, RvD1 and RvE1 were shown to promote resolution of inflammation in microglial cells in vitro (Rey et al., 2016). ChemR23/ERV1 is also expressed by retinal microglia and plays a role in protecting pathological retina angiogenesis by inhibiting TNF production (Connor et al., 2007). In a mouse model of traumatic brain injury, RvE1 treatment increases post-traumatic sleep and normalized microgliosis in the injured brain (Harrison et al., 2015). RvE1 has also been shown to alleviate neuropathic pain by inhibiting microglial activation in intact spinal cord and TNF release in microglia cultures (Xu et al., 2013a). Future studies are necessary to test whether these actions are mediated by the ChemR23 receptor. RvD1 reduces the activation of microglia and improves functional recovery after focal brain damage via the murine ALX/FPR2 receptor (Bisicchia et al., 2018). Spinal treatment of LXA4 attenuated neuropathic pain and spinal microgliosis and TNF expression after spinal cord injury. Furthermore, microglia express ALX/FPR2 which appears to mediate LXA4-induced inhibition of TNF release from microglia (Martini et al., 2016). Kantarci et al. reported decreased levels of SPMs in the hippocampus of mice with Alzheimer's disease (AD), but a combined treatment of RvE1 and LXA4 decreased neuroinflammation (microglia and astrocyte activation) associated with Aβ pathology in AD mice (Kantarci et al., 2018).

Emerging Role of Microglia in Chronic Itch

Pain and itch (pruritus) are distinct sensations, leading to respective withdrawal response following noxious stimuli and scratching response following pruritic stimuli. Pain is also known to suppress itch, while analgesics such as morphine evokes itch after spinal delivery (LaMotte et al., 2014). Great progress has been made in elucidating itch's neuronal mechanisms, including peripheral and central mechanisms of itch (Dong and Dong, 2018; Sun and Chen, 2007). Chronic itch is associated with benign skin lesions or cancers such as lymphoma (Han et al., 2018; Yosipovitch and Bernhard, 2013). Distinct mechanisms have been implicated in acute itch and chronic itch(Liu and Ji, 2013). Increasing evidence suggests a role for spinal microglia in the pathogenesis of chronic itch. For example, inhibition of spinal microglial signaling with minocycline and TLR4 antagonist reduced dry skin-evoked chronic itch, without affecting acute itch (Liu et al., 2016). Spinal inhibition of TNF also reduced chronic pruritus (Miao et al., 2018). These studies are still preliminary, and further investigation of microglial signaling in chronic itch in both sexes is necessary.

Clinical Relevance and Future Directions

The clinical relevance of microglial pathophysiology has been challenging to establish, despite the plethora of animal research. The earliest direct indication of microglial activity in human pain states appears to be immunohistochemical data where post-mortem spinal cord from patients with herpetic neuralgia was noted to have microgliosis in the dorsal horns that mapped to the dermatomal region of the rash (Denny-Brown, 1944). Loggia et al were able to verify thalamic glial activation occurred in chronic low back pain patients using positron emission tomography (PET) imaging (Loggia et al., 2015). However, the clinical translation of available microglial modulatory agents has not had robust or consistent results in trials

(Curtin et al., 2017; Martinez et al., 2013; Sumitani et al., 2016; Sumracki et al., 2012; Vanelderen et al., 2015; Younger and Mackey, 2009). A pilot study revealed that fibromyalgia symptoms are reduced by low-dose naltrexone (Younger and Mackey, 2009), as low-dose naltrexone was shown to inhibit microglial activation similar to TLR4 inhibitor (Wang et al., 2016). A small trial revealed that minocycline does not decrease intensity of neuropathic pain intensity, but does improve its affective dimension (Sumitani et al., 2016). In a recent clinical study, Curtin tested whether perioperative administration of minocycline would improve pain resolution after standardized hand surgeries in patients with carpal tunnel syndrome, a neuropathic pain condition. Oral administration of minocycline did not reduce time of pain resolution after minor hand surgery. In contrast, there was evidence that minocycline might increase length of pain in those with increased posttraumatic stress disorder symptoms (Curtin et al., 2017), suggesting a possible a role of microglia in promoting pain resolution. In a randomized, double-blind, controlled study, Martinez et al. randomly assigned 100 patients undergoing scheduled lumbar discectomy to placebo and minocycline groups (100 mg orally, twice daily, beginning before surgery and continuing for 8 days). The results showed that perioperative minocycline fails to improve persistent pain after lumbar discectomy. However, exploratory analysis suggested that minocycline might be effective in a subgroup of patients with predominantly deep spontaneous pain at baseline (Martinez et al., 2013). In another randomized, double-blind, placebo-controlled clinical trial, patients with subacute lumbar radicular pain received placebo, amitriptyline 25 mg (which was shown to inhibit spinal p38 activation and neuroinflammation in animal model (Tai et al., 2009), or minocycline (100 mg once a day, n = 20 per group) for 14 days. Minocycline produced no side effects, whereas amitriptyline produced side effects in 10% of patients. Both treatment groups significantly differed from placebo group, but their effect size in pain reduction was small (Vanelderen et al., 2015). There could be several reasons underlying the mixed results and lack of efficacy. First, there are no biomarkers to demonstrate that the treatments i) engage microglia in the spinal cord and brain and ii) alter microglial activity and microglia-mediated neuroinflammation. Second, there could be non-specific (non-microglial) effects of the agents studied, such as minocycline or naltrexone which have pleotropic effects including on metalloproteases and opioid receptors respectively(Ji et al., 2009). Third, there is lack of full understanding of sex dimorphism in microglial pathophysiology in human trials (Berta et al., 2016; Sorge et al., 2015). Fourth, there is lack of appreciation of distinct role of microglia in early vs. late phase clinical pain and a protective role of microglia in the resolution phase (Ji et al., 2009). The lack of robust or consistent effects of microglial modulators clinically may also be due to the study design and heterogeneity of the patient populations, or due to underlying biology. It is noted for example, in postmortem spinal cord tissues from patients with HIV neuropathy, that microgliosis is less obvious than astrogliosis, suggesting perhaps astroglial signaling may be more critical for chronic human pain (Shi et al., 2012).

Microglial biology has been somewhat hampered by the tools and techniques we have to study these powerful but mutable cells. Unlike many of the glial or neuronal cells in the CNS, microglia can rapidly proliferate and change in response to stimuli, while still being maintained for years to decades. New and specific antibodies to identify microglia specifically rather than peripheral macrophages, in combination with new microscopy and

tissue preparation methods, should allow for visualizing microglia in vivo or in vitro without disturbing their interactions with surrounding cells and provide better insights into the microglial regulatory framework in pain regulation. With the paucity of specific modulators for microglia, chemogenetic techniques such as DREADDs will also allow for better insights into the role of microglia in various pain states (Grace et al., 2016; Grace et al., 2018). Most exciting, however, is the promise of single-cell based assays that would allow for evaluation of the unique transcriptomes or proteomes of microglia in various physiologic and pathologic states (Ajami et al., 2018; Mathys et al., 2017; Mrdjen et al., 2018). The appreciation that microglia are not a monolithic homogenous cell type, but rather a spectrum of actively changing cells with unique transcriptional and proteomic patterns, should allow us to better understand their role in different pain states (De Biase et al., 2017; Dukhinova et al., 2018; Furube et al., 2018). Application of techniques such as mass cytometry or single-cell sequencing in various models of pain, at various time points, as well as on the resident microglia in pain circuits should help us develop deeper understandings into their spatiotemporal activation parameters.

Although direct targeting of microglial activation and neuroinflammation via inhibitors of microglia, TLRs, ATP receptors, cytokines, and MAP kinases could be effective, these drugs may also produce side effects such as infection and impair the resolution of inflammation, especially after long-term treatment. We should also consider pro-resolution strategies that can control abnormal microglial activation and excessive neuroinflammation as well as promote a return to homeostasis, such as pharmacological approaches (e.g., SPMs and CB2 agonists) (Fig. 4), cell therapies (e.g., bone marrow stem cells), and neuromodulation (e.g., spinal cord stimulation)(Chen et al., 2015; Ji et al., 2018; Sato et al., 2014).

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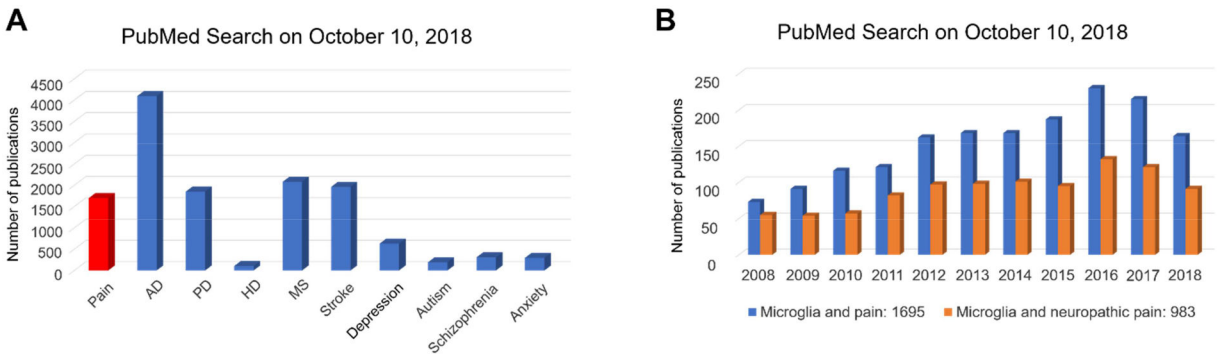

Figure-1: PubMed search for microglia and neurological diseases.

(A) Number of publications involving microglia in pain, Alzheimer's disease (AD), Parkinson disease (PD), Huntington disease (HD), multiple sclerosis (MS), stroke, depression, autism, schizophrenia, and anxiety. **(B)** Number of publications for microglia and pain and microglia and neuropathic pain in last 10 years.

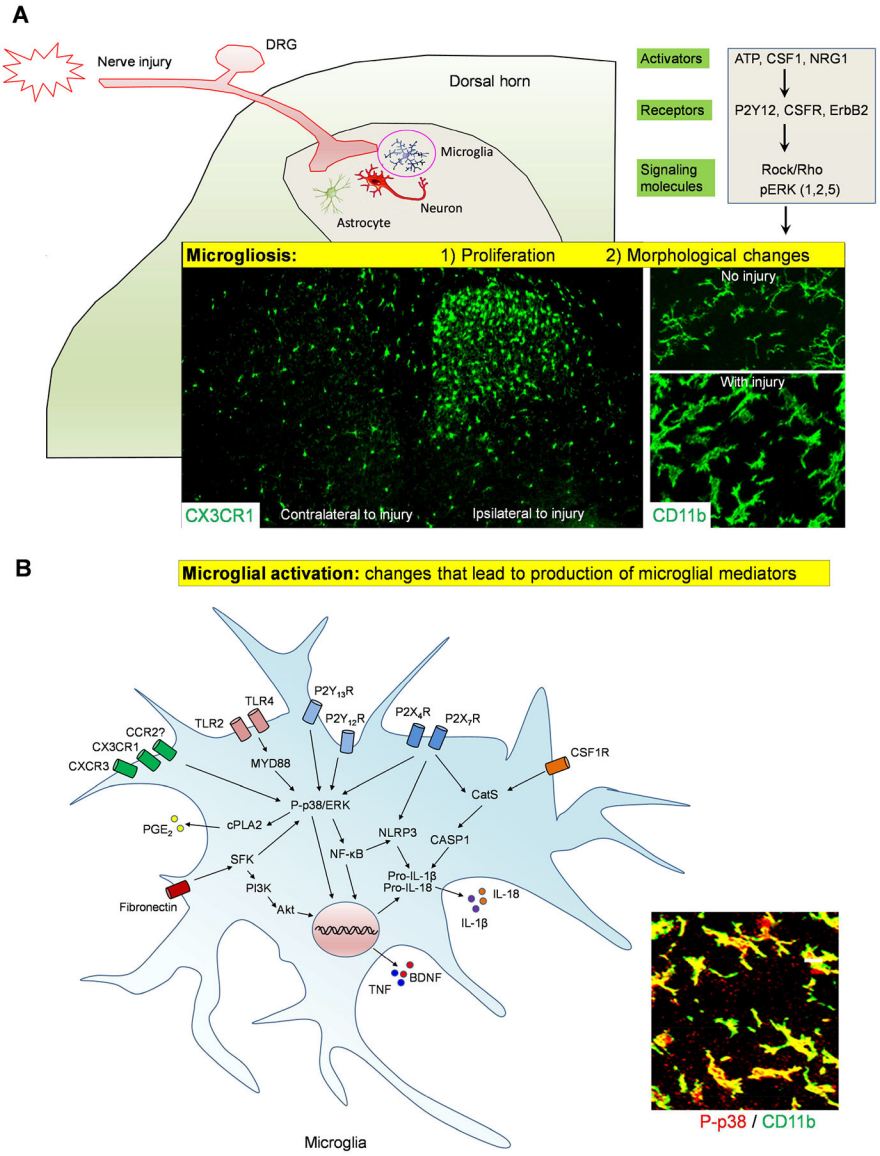

Figure-2: Nerve injury-induced microgliosis and microglial activation in the spinal cord.

(A) Microgliosis in SDH after nerve injury. (1) Nerve injury (CCI) induces microglial proliferation on the ipsilateral side as revealed in CX3CR1-GFP mice. (2) Nerve injury also induces profound morphological changes of microglia as revealed by CD11b staining with OX-42 antibody. (B) Microglia activation after nerve injury results in the production of microglial mediators, such as TNF, BDNF, IL-1β, and IL-18, which can promote neuropathic pain.

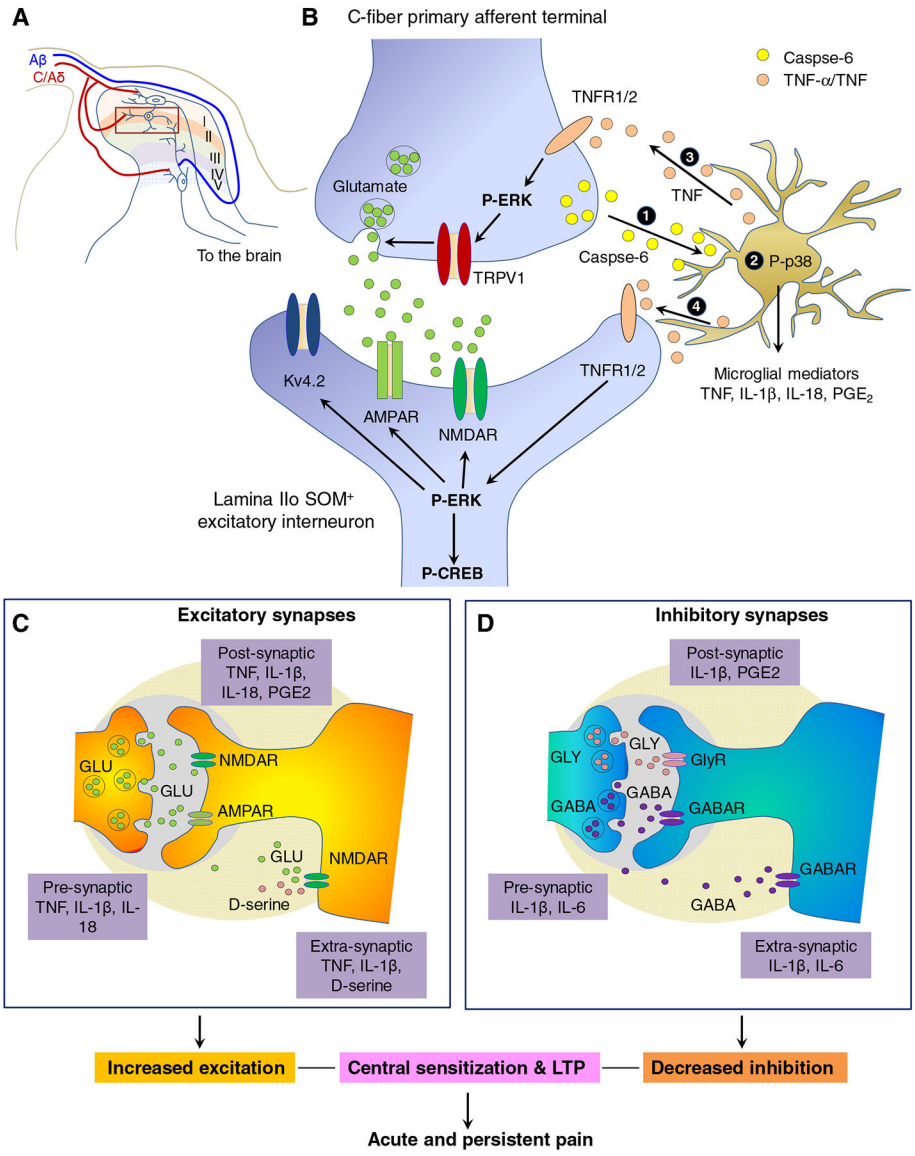

Figure-3:

Schematic illustration of microglial regulations of acute and persistent pain in spinal cord pain circuit via multiple mechanisms, including presynaptic mechanisms and post-synaptic mechanisms and regulations of both excitatory and inhibitory synaptic transmission.

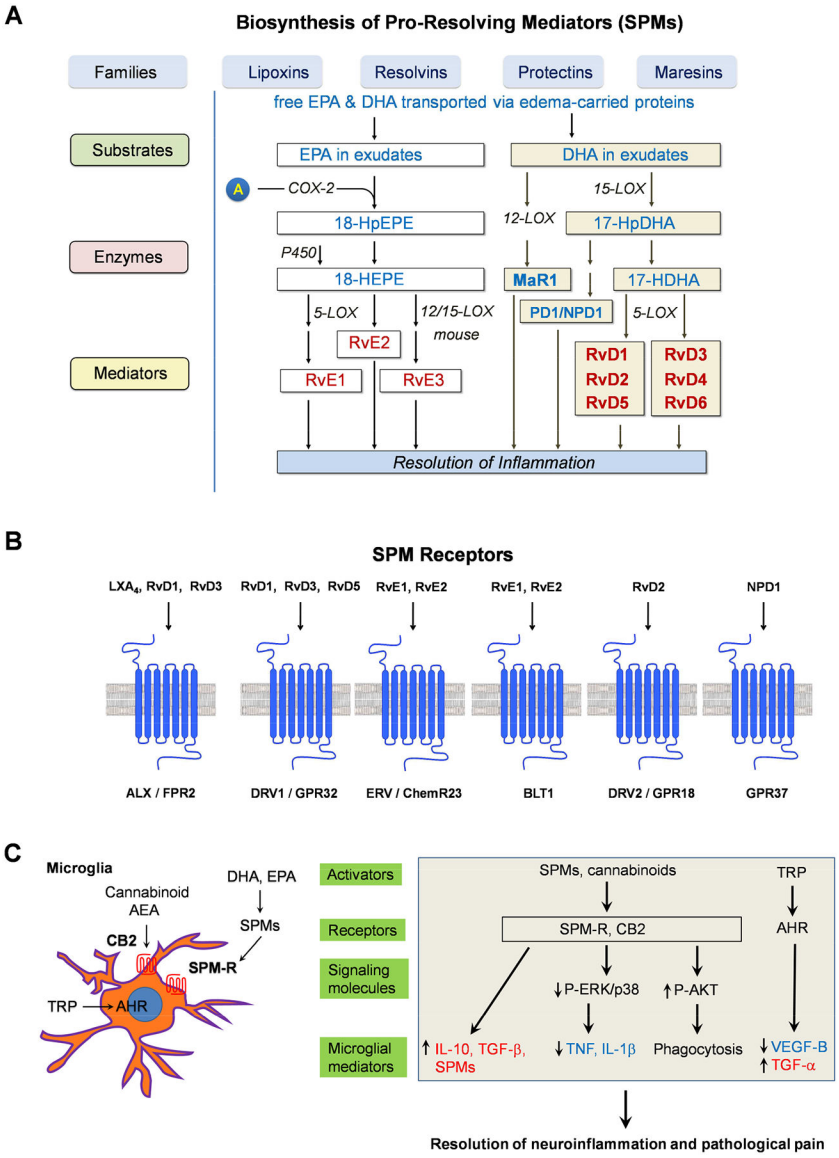

Figure 4: Schematic for microglial regulation of the resolution of neuroinflammation.

(A) Biosynthesis of SPM families of lipoxins, resolvins, protectins/neuroprotectins, and maresins from DHA and EPA. (B) SPMs receptors. ChemR23 (RvE1 receptor), GPR32 (RvD1 receptor), and GPR18 (RvD2 receptor) are denoted ERV, DRV1, and DRV2, respectively, as with the system of nomenclature for eicosanoid receptors). (C) Microglial signaling via SPM receptors, CB2, and aryl hydrocarbon receptor (AHR), in response to their ligands such as SPMs, cannabinoid, and gut-derived metabolites such as tryptophan (TRP). Activation of these microglial receptors enhances phagocytic activity, reduces the expression of pro-inflammatory mediators (e.g., TNF, IL-1β, and VEGF-B), and increases the production of anti-inflammatory mediators (e.g., IL-10, TGF-α, TGF-β) and SPMs, thus promoting the resolution of neuroinflammation and pathological pain.

Table-1:

Microglial signal molecules that are upregulated after peripheral nerve injury and positively contribute to promoting neuropathic pain (mechanical allodynia).

Upregulation of signaling molecules	Nerve injury models	Contribution to mechanical allodynia	References
Purinergic receptors			
P2X4R	SNT	Yes	Tsuda et al., 2003
P2X7R	SNI	Yes	Kobayashi et al., 2011
P2Y6R	SNI, SNL	Yes	Kobayashi et al., 2012; Barragan-Iglesias et al., 2014
P2Y12R	SNL, SNT	Yes	Tozaki-Saitoh et al., 2008; Kobayashi et al., 2008
P2Y13R	SNI	Yes	Kobayashi et al., 2012; Tatsumi et al., 2015
P2Y14R	SNI	Yes	Kobayashi et al., 2012
Toll-like receptors			
TLR-2	SNT	Yes	Kim et al., 2007
TLR-4	SNT	Yes	Tanga et al., 2005
Chemokine receptors			
CX3CR1	SNL	Yes	Zhuang et al., 2007; Staniland et al., 2010
CCR2	SNL	Yes	Abbadie et al., 2003
CCR5	SNT	Yes	Matsushita et al.,2014
Other receptors			
TREM2	SNT	Yes	Kobayashi et al., 2016
ErbB2	SNI	Yes	Calvo et al., 2010
TMEM16F	SNL	Yes	Batti et al., 2016
C5aR	SNI	Yes	Griffin et al., 2007
IFN-γR	SNI	Yes	Tsuda et al., 2009
CSF1R	SNI, SNT	Yes	Guan et al., 2016; Okubo et al., 2016
Kinases and enzymes			
p-p38	SNL,SNT	Yes	Jin et al., 2003; Tsuda et al., 2004
pERK1/2	SNL	Yes	Zhuang et al., 2005
pERK5	SNL	Yes	Obata et al., 2007
pSrc	SNT	Yes	Katsura et al., 2006
PLyn	SNT	Yes	Tsuda et al., 2008
Nox-2	SNT	Yes	Kim et al., 2010
COX-1	SNT	Yes	Kanda et al., 2013
COX-2	SCI	Yes	Zhao et al., 2007
5-LOX	SNI	Yes	Okubo et al., 2010
PGDS	SNT	Yes	Kanda et al., 2013
TXA2S	SNT	Yes	Kanda et al., 2013
LPCAT2	SNL	Yes	Shindou et al., 2017
Cathepsin S	SNL	Yes	Clark et al., 2007
Transcriptional factors			
IRF8	SNT	Yes	Masuda et al., 2012
IRF5	SNT	Yes	Masuda et al., 2014

Upregulation of signaling molecules	Nerve injury models	Contribution to mechanical allodynia	References
STAT3	SNL	Yes	Dominguez et al., 2008
Other proteins			
C1q, C3,C4,C5	SNL, SNI, CCI	Yes	Griffin et al., 2007
ROS	SNT	Yes	Kim et al., 2010
DAP12	SNT	Yes	Guan et al., 2016; Kobayashi et al., 2016

Abbreviations: CCI, chronic constriction injury; SNI, spared nerve injury; SNL, spinal nerve ligation; SNT, spinal nerve transection

Table-2.

Sex dimorphism in spinal microglial signaling in pathological pain after peripheral nerve injury (PNI), hind paw injection of formalin and complete Freund's adjuvant (CFA), and bone cancer. N.T., not tested.

	Sex differences	Male	Female	References
Microgliosis				
Proliferation	No (PNI)	Yes	Yes	Sorge et al., 2015; Taves et al., 2016
Morphological changes	No (PNI)	Yes	Yes	
Iba1/CX3CR1 expression	No (PNI)	Yes	Yes	
TMEM16F	No (PNI)	Yes	Yes	Batti et al., 2016
Microglial ablation (Mac-1-SAP)	Yes (PNI)	Yes	No	Sorge et al., 2015
Microglia activation & signaling				
Minocycline (intrathecal)	Yes (PNI, Formalin)	Yes	No	Sorge et al., 2015; Chen et al., 2018
	Yes (Bone cancer, SCI)	N.T.	Yes	Chen et al., 2012; Yang et al., 2015
TLR4 inhibitor	Yes (PNI, CFA)	Yes	No	Sorge et al., 2011
P-p38 expression & p38 inhibitor	Yes (PNI)	Yes	No	Taves et al., 2016
	Yes (hyperalgesic priming)	Yes	No	Paige et al., 2018
	Yes (Bone cancer)	N.T.	Yes	Yang et al., 2015
p38-a knockdown	Yes (PNI)	Yes	No	Luo et al., 2017
Caspase-6 inhibitor	Yes (PNI)	Yes	No	Berta et al., 2017; Chen et al., 2018
P2X4/BDNF expression & inhibitor	Yes (PNI)	Yes	No	Sorge et al., 2015