Austin Journal of Clinical Neurology

Open Access

Austin Publishing Group

Review Article

Research Progress of CCL2-CCR2 Signaling in Neuropathic Pain

Ying Zhang*
Department of Anesthesiology, Hospital (TCM) Affiliated To Southwest Medical University, China

***Corresponding author: Ying Zhan**
Department of Anesthesiology, Hospital (T.C.M) Affiliated to Southwest Medical University, Luzhou, China.
Email: zhangying021210@163.com

Received: April 29, 2023
Accepted: May 22, 2023
Published: May 29, 2023

Abstract

Neuropathic Pain (NPP) is a chronic disorder of maladaptation that may occur after an injury or disease which affects the somatosensory system and features as abnormal pain, nociceptive hyperalgesia, spontaneous pain, and comorbidity triggering sleep disorders, depression, and anxiety. Despite recent advances and growing interest in understanding the pathogenesis of this disease, neuropathic pain remains a significant challenge to treat. Studies have shown that neuroinflammation has a significant function for the establishment and the development of neuropathic pain. The chemokine CCL2 mediates responses to both inflammation and immune responses within the nervous system, and new data suggests that CCL2/CCR2 signaling performs a significant function in neuroinflammation by participating in neuron-glial cell interactions. Here, we provide evidence regarding the contribution of CCL2/CCR2 in neuropathic pain. First, we reviewed the mechanisms whereby CCL2-CCR2 promotes plasticity changes in neuropathic pain conditions from three different anatomical sites: the nerve injury site, the dorsal horn of the central spinal cord, and the dorsal root ganglion. Then, we report on the study regarding CCL2-CCR2 as a potential therapeutic target for neuropathic pain: Can CCL2-CCR2 induce neuropathic pain by bridging peripheral and central neuroinflammation through altering the permeability of the Blood-Spinal Cord Barrier (BSCB)?

Keywords: Neuropathic pain; CCL2/CCR2; Neuroinflammation

Introduction

Pain is an unpleasant experience of the body to the damaged tissue or potential injury, which is a complex physiological and psychological activity, as one of the most common clinical symptoms [1]. Therefore, Neuropathic pain is a presentation of common signs and symptoms that may originate in the peripheral and central nervous system, rather than a disease [2]. The perception of pain is usually associated with an inflammatory response, in which the body responds to tissue damage caused by disease and lesions with a complex biological response involving in recruiting of immune cells and the release of mediators that have adverse effects such as sensitization and stimulation of nociceptor, leading to increased central synaptic transmission. In 2014 Ji et al. fully reviewed the vital function of neuroinflammation in the osteogenesis of chronic pain [3]. Neuroinflammation can result in the release of inflammatory mediators like pro-inflammatory cytokines, growth factors as well as chemokines by glial cells acting on neurons in both the periphery and spinal cord [4-7]. In addition, inflammatory mediators (including chemokines) also can be released from primary afferent neurons or spinal cord neurons to induce activation of glial cells [8-10]. In recent years, an increasing number of researches reveal significance of chemokines in the context of chronic pain: Among inflammatory mediators, chemokines are proven to be associated with peripheral sensitization and central sensitization following nerve injury [11], from which both are contributors to the development and maintenance of neuropathic pain [3,12,13]. However, despite intensive research on the nosogenesis of neuropathic pain, the mechanisms that underlie it await elucidation, and there are currently no validated for neuropathic pain. Therefore, the exploration of novel molecular mechanisms participating in Neuropathic pain may facilitate the discovery and development of promising analgesic drugs, which is therefore eagerly anticipated. Recently, chemokine CCL2 has been considered as a significant new player in

Citation: Zhang Y. Research Progress of CCL2-CCR2 Signaling in Neuropathic Pain. Austin J Clin Neurol. 2023; 10(1): 1160.

pain control for contributing to the development of neuropathic pain after nerve injury. Moreover, a growing body of evidence supports the contention that CCL2 has significant contributions to the progression of neuropathic pain. Here, we discuss evidence from studies on the pathogenesis of the promotion of neuropathic pain by the upregulated chemokine CCL2 with its receptor CCR2 at different anatomical sites (nerve injury site, Dorsal Root Ganglion (DRG), and central spinal cord dorsal horn) after peripheral nerve injury. We then discuss possible mechanisms of the CCL2-mediated progression of neuropathic pain through the blood-spinal cord barrier.

CCL2\CCR2 AND NPP

Chemokines are a small class of signaling proteins secreted by cells of the immune system, which are named due to their responsibility in regulating the activity of other cells to respond to chemical stimuli (chemotaxis). Based on the location of N-terminal cysteine residues, the chemokines are classified into four major families: two major subgroups CXC and CC, and two minor subgroups CX3C and C [14].

Functional expression of all chemokines is achieved by binding to their corresponding cell surface receptors (GPCRs) and activating intracellular signaling cascades, including the MAPK pathway, the PLC pathway, and the PI3K pathway [15], producing biological properties such as adhesion, polarization, as well as chemotaxis. However, there is no a one-to-one correspondence between chemokines and receptors; a chemokine can bind to one or more chemokine receptors. And CCL2, a member of the CCs family, also known as MCP-1, was first identified in 1989 [16] and, together with its major receptor CCR2, is known to be one of the most prominent neuroactive chemokine pairs regulating nociception ability, as well as a critical chemokine pairs moderating the migration and infiltration of monocytes/macrophages [17-20]. CCR2 is the preferred receptor for CCL2, in addition to CCR1, CCR2, and CCR4, which are recognized by CCL2 [21].

In 2015, Tracy et al. proposed that the CCL2-CCR2 signaling is associated with some inflammatory responses and diseases, one of which includes neuropathic pain [22,23]. Besides, recently, there is also a growing piece of evidence affirming the important function of the CCL2/CCR2 signaling in the nociceptive production and transmission, particularly in the treatment of neuropathic pain [19,20,24-26]. For example, Takahiro et al. induced a sustained painful mechanical hypersensitivity response by intrathecal injection of CCL2 in 2004 [27-29], and Marc-André, Michael et al. verified these findings in 2008 and 2009; it has also been reported that CCR2 blockade can eliminate nociceptive hypersensitivity in various rodent models of neuropathic pain [6,29-31], such as Abbadie et al. in 2003, who demonstrated that mice knocked out of the CCR2 gene do not develop mechanical nociception in a CCI model [32-34]. Not only that, substantial evidence suggests that chemokine CCL2 can produce inflammatory responses in different nerve sites, including injured nerves, dorsal root ganglia, spinal cord, etc., from which it contributes to the management of chronic pain [25,35-38]. Therefore, we explored the function of the CCL2-CCR2 signaling pathway in the pathogenesis of neuropathic pain according to different nerve sites.

Role of CCL2/CCR2 Signaling in PNS

Macrophages are critical in the peripheral nervous system after nerve injury—neuroinflammation characterized by Wallerian degeneration intermediates the onset of pain after peripheral nerve injury, in which macrophages participate the removal of myelin debris, something that is essential for the resolution of inflammation. In 1993, Griffin et al. demonstrated that within the majority of tissues, of which the nervous system is a part, macrophages can be distinguished into two main categories: resident macrophages, which exist under steady-state conditions, and infiltrating macrophages, which infiltrate into tissues as a result of injury or infection [39]. Infiltrating macrophages, which originate from monocytes in the bone marrow, can migrate to sites of damage or infection [40], and in 2003 Mueller et al. found remarkable increases in infiltrating macrophages on the seventh day after sciatic nerve transection with a ratio of infiltrating to resident macrophages of approximately 3:1 [41], demonstrating the migration of infiltrating macrophages. Martinez et al. in 2014 demonstrated that infiltrating macrophages arriving to the sites of nerve injury can differentiate into the pro-inflammatory M1 type [42]. These infiltrating M1-type macrophages further remove myelin debris through phagocytosis, while releasing large amounts of inflammatory mediators like IL-1β, IL-6, and TNFα to promote inflammation, leading to increased peripheral sensitization and pain [43,44].

So what are the upstream signaling molecules that can induce monocytes/macrophages to migrate to injured tissues to function? Recent evidence suggests that the chemokine CCL2 is an important new player in pain contro. Numerous experimental data validate the above arguments, Niemi and Siebert in 2013 and 2000 found a reduction of macrophage recruitment to the remote sciatic nerve and its DRG on the modeling side after the knockdown of CCL2 or CCR2 [39]. In addition, Lindborg et al. in 2017 demonstrated that the use of antibodies against CCR2 leads to a decline in both blood and sciatic nerve accumulation of monocytes/macrophages in blood and sciatic nerve [45]. As the above arguments illustrate the relevance of CCL2/CCR2 signaling to macrophages in response to nerve injury, we further discussed the mechanism of CCL2/CCR2 signaling that mediates peripheral sensitization leading to pain on the example of the model of sciatic nerve injury.

It is well known that Wallerian degeneration occurs rapidly in response to nerve injury correlated with monocyte/macrophage infiltration of the damaged nerve. Wallerian degeneration refers to a typical series of changes which occur after nerve fiber injury, including macrophage invasion, Schwann cell activation, and upregulation of neurotrophins and cytokines, and in 2011 Dubový demonstrated that the above-mentioned changes are responsible for both axon growth and the induction of neuropathic pain [46]. Besides, Dubový also demonstrated that Wallerian degeneration is also connected with an inflammatory response, mainly involving the immediate upregulation of immune signaling elements like cytokines or chemokines factors, with this in turn leads to inflammation and persistent nociceptive hyperalgesia and allodynia [43].

CCL2, one of the most characterized chemokines that bring monocytes into tissues, is mainly upregulated in Schwann cells following nerve injury [47] as well as mediating the movement of pro-inflammatory monocytes to sites of tissue with inflammation, acting upon binding to CCR2 (CCR2 is mainly expressed by monocytes and macrophages) [31,48]. In 1997, Bruck et al. demonstrated that in the SNI state CCL2 drives the export of mononuclear myeloid cells to the vasculature before infiltrating into inflammatory tissues and later differentiating into macrophages and participating in Wallerian degeneration [49]. At

the same time, Wallerian degeneration is thought to relate to increases in pain behavior. It was found that when the time for macrophage recruitment exceeds the time necessary for tissue repair, then it would lead to tissue damage and the release of inflammatory mediators which further exacerbate pain.

In summary, after nerve injury, the upregulated CCL2 in Schwann cells mediates the infiltration of monocytes into the site of nerve injury by binding to CCR2 on monocytes and differentiating into macrophages, which are involved in axonal degeneration of Wallerian degeneration, which itself increases pain behavior and can be considered as a potential mechanism for exacerbating peripheral sensitization. Also migrating macrophages themselves release large amounts of inflammatory factors leading to peripheral sensitization, which is one of the other mechanisms involved in peripheral sensitization. Thus, the CCL2/CCR2 signaling coordinates the neuroinflammatory response and thus promotes pain production mainly through the recruitment of monocytes [20,24] (Figure 1).

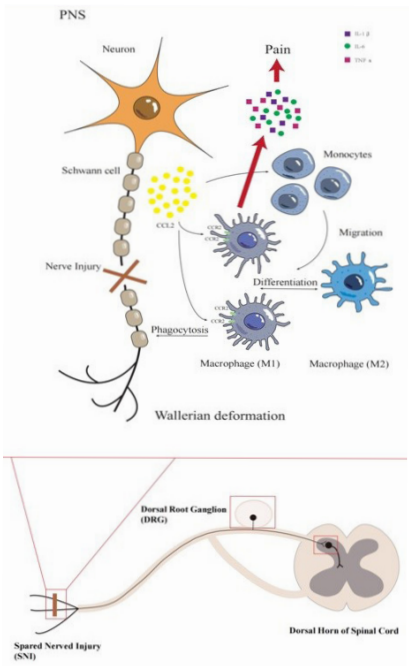

Figure 1: Schematic representation of the role of CCL2/CCR2 after peripheral nerve injury.

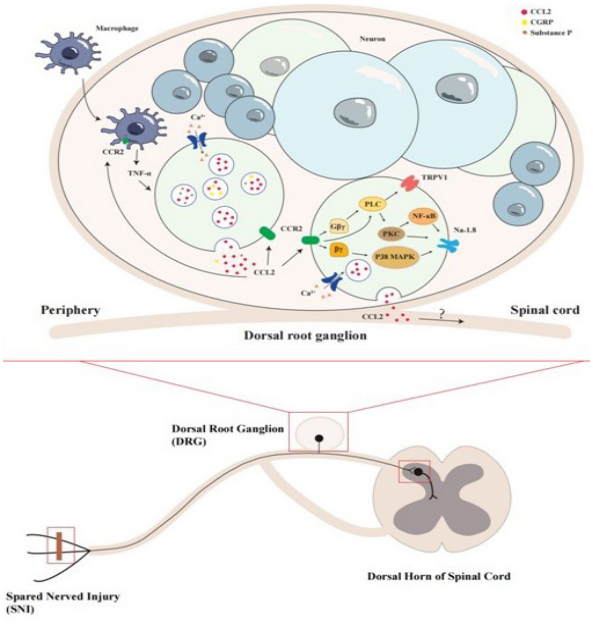

Figure 2: Schematic representation of the role of CCL2/CCR2 in the dorsal root ganglion.

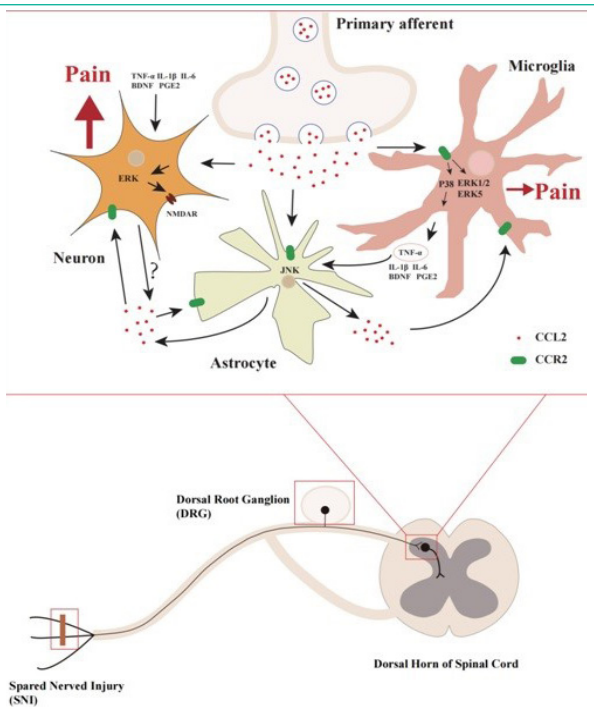

Figure 3: Schematic representation of the role of CCL2/CCR2 in the spinal cord.

Role of CCL2\CCR2 Signaling in DRG (Dorsal Root Ganglion)

The DRG is related to the onset and maintenance of neuropathic pain [50],

which is a swollen junction formed by the dorsal roots of the spinal cord, distributed throughout the spinal cord on both sides of the medial intervertebral foramina, with a total of 31 pairs, housing the cell bodies of primary sensory neurons, and every DRG is capable of holding up to 15,000 neurons, and depending on the diameter and origin of the neurons, the neurons in the DRG can be divided into small, medium and large neurons, regulating the impulse transduction from the periphery, including pain perception [51]. In 2002, Xiao et al. demonstrated that axotomy of rat DRG neurons leads to various changes in molecular expression, including receptors, ion channels, and synaptic vesicle proteins, moreover, Xiao suggested that cascade expression of these genes may be a trigger for neuropathic pain [52]. Later Krames et al. proposed in 2014 that upregulation of ion channel expression and subsequent inflammatory responses in response to nerve injury probably characterize chronic pain [50,53], and Liem et al. also proposed in 2016 that nerve injury can lead to functional changes such as ion channel expression which further leads to peripheral sensitization of DRG neurons by overexcitation resulting in neuropathic pain [53,54]. Not only that, the function of DRG in neuropathic pain is increasingly acknowledged within recent years which is considered to promote pain delivery and persistent state, so exploring the pain mechanism of DRG is one of the ways to find a targeted treatment for pain.

CCL2 and CCR2 do not express in DRG neurons of healthy animals [55], whereas in 2007 Hosung et al. demonstrated the up-regulation of CCL2 and CCR2 expression in DRG neurons in pathological situations. Not only that, but later an increasing number of studies found that CCL2 and CCR2 expression was upregulated in DRG in multiple rodent models of neuropathic pain [55-58], including sciatic nerve dissection [59,60], partial sciatic nerve ligation [61], Chronic Constriction Injury [62-64], Chronic Compression of the L4L5 DRG (CCD), spinal stenosis [65], bone cancer pain [66,67] and yeast polysaccharide-in-

duced inflammatory pain [58,68]. Zhang et al. [12] found that following nerve injury, small to large-diameter DRG neurons express ATF-3, a marker of nerve injury, in addition to upregulated CCL2 expression, thus suggesting that the expression of CCL2 rises in damaged neurons, and they also demonstrated that CCL2 can directly stimulate sensory injured neurons through autocrine and/or paracrine processes [63].

Hosung et al. demonstrated that CCL2 upregulation in DRG neurons can be achieved through the cytokine TNF-α [69], whose potential sources include cells relevant to sites of peripheral nerve injury and neuroglia cells (microglia and astrocytes) in the central projection region of primary afferent neurons in the spinal cord [70,71]. Thus, following nerve injury, TNF-α can operate as an upstream factor regulating chemokine transmission pathways. Similarly, there is relevant confirmation regarding the upregulation of CCR2 expression, for example, in 2006 Sun et al. proved that cultured DRG neurons in vitro express multiple chemokine receptors containing CCR2, of which activation excites sensory neurons and produces hyperesthesia [65].

After nerve injury, it is synthesized of CCL2 in neurons in the DRG which can be contained in vesicles containing and not containing neuropeptides. In 1996 Huang et al. found that neuropeptides located in vesicles of DRG neurons are secreted from the neuronal cytosol through Ca2+-dependent cytokinesis [72], for example, substance P and CGRP, while Ouyang et al. verified these findings in 2005 [73,74]. Whereas CGRP and substance P were shown to be proinflammatory molecules associated with pain sensitization by Hosung et al. [75], therefore CCL2-induced excitation may coincide with the release of these pain-related neurotransmitters [75,76]. It was found that CCL2 is synthesized in cultured DRG neurons and then packaged into synaptic vesicles identical to the peptide neurotransmitter CGRP. Depolarization of neurons results in the release of CGRP from somatic cells or nerve endings in a Ca2+-dependent way, while the chemokine CCL2 can also be released to further sensitize neurons, proving the above point [38]. In summary, the release of CCL2 from DRG neurons can depolarize and generate hyperexcitability in the same neuron or neighboring CCR2-expressing neurons, promoting the further release of CCL2 within the DRG and creating positive feedback. The released CCL2 continues to transmit pain-related information after binding to CCR2-expressing neurons or glial cells. Thus, CCL2/CCR2 signaling may be a significant trigger for DRG hyperexcitability and maintenance of chronic pain.

We believe that CCL2 results in the generation of neuropathic pain by upregulating neurotransmitters which promote peripheral sensitization and indirectly overexcite sensory neurons. So, what is the molecular mechanism whereby CCL2 mediates the generation of excitation? Many molecular sensors on primary nociceptive receptors, for instance, GPCRs, TRP, and sodium channels (e.g., Nav1.8) [3,77], may be involved in the formation of excitation. Oh and White, Jung et al. demonstrated that released CCL2 acts on CCR2 expressed on the membranes of nociceptive DRG neurons, which consequently causes strong activation of nociceptive neurons [57,75,78], so the excitation of these neurons implicates the ion channels mentioned above as possible downstream targets of CCL2/CCR2 signaling. There are a large number of experiments that prove the above point, such as Kao et al. who demonstrated that CCL2 upregulates the Nav1.8 current density and TRPV1 expression among DRG neurons [79]. Also, Ruparel et al. proved that neuronal hyperexcitability may be related to the transactivation of TRP like TRPV1 and TRPA1 expressed in the injurious neuronal population [80,81]. Not only that, but evidence has also been proposed to be related to voltage-gated sodium channels in nociceptive neurons [38]. Therefore, we provide a detailed description of several of these arguments.

First, it was shown that released CCL2 can sensitize nociceptors through the transactivation of TRP channels upon binding to CCR2, where sensitization triggered by TRP channels appears to be responsible for pain hypersensitivity [72]. TRPV1 is the main TRP channel that participates in cold and heat perception and nociceptors sensitization which can be triggered by painful stimuli and some inflammatory mediators [82]. TRPA1 is another TRP channel that has an important function in nociception and participates in pain. Recently, Kao et al. demonstrated that increased CCL2/CCR2 signaling strengthens and upregulates the function as well as expression of TRPV1 ion channels, causing peripheral sensitization of DRG injurious neurons and driving nociceptive hypersensitivity states [34,75,79]. Also, Van et al. demonstrated in vitro perfusion experiments that capsaicin induces the calcium-dependent release of CCL2 [28], while Spicarova demonstrated that TRPV1 inhibition reduces CCL2-induced nociceptive sensitization [83]. Thus, in summary, it can be concluded that the CCL2/CCR2 signaling may lead to the hyperexcitability of DRG neurons via the TRP pathway.

Secondly, Voltage-Gated Sodium Channels (VGSC) have a vital effect on the electrophysiological onset and impulse conduction, which may therefore be responsible for the development of peripheral hyperexcitability in neuropathic pain conditions. Nav1.8 is one of the subtypes of VGSC, preferentially expressed in primitive sensory neurons, that upon stimulation, generates slowly inactivating Nav1.8 currents and accounts for action potential propagation in sensory neurons of the DRG. It is found that CCL2 concentration dependently increased Nav1.8 current density in small and medium-diameter sensory neurons, which was reversed by the CCR2 antagonist INCB [84]. Lampert et al. demonstrated that functional knockdown or knockout of the gene expressing Nav1.8 in rats reduced hyperalgesia in models of neuropathic pain. Similarly, Bear and Matulenko et al. found that systemic or intrathecal injection of Nav1.8 blockers reversed a portion of pain manifestations in preclinical animal models of chronic pain [85-88]. At the same time, numerous studies have also found increased reactivity of neurons expressing Nav1.8 that would lead to the generation of nociceptive pain hypersensitivity, hyperalgesia, and persistent spontaneous pain [89,90]. As such, Nav1.8 is considered the key reason for the enhanced excitability and spontaneous ectopic discharge of medium-sized and small neurons in the DRG [91-93], and may also be the pathogenesis of primary sensory neurons mediating chronic pain.

Several types of researches have been reported to demonstrate the participation of CCL2/CCR2 in regulating the manifestation and ampere density of Nav1.8 in DRG neurons [79,94], Currently, several different potential mechanisms are suggested on how pro-inflammatory mediators increase Nav1.8 currents as well as enhance the excitability of nociceptors via GPCR (G protein-coupled receptor) [88,90]. CCR2, as one of the basic GPCRs, couples to the Gα and Gβγ subunits of trimeric G proteins to activate many signaling pathways, including PLC, ERK1/2, and p38 MAPK [26,95], while the Nav1.8 channel has been described as the ultimate co-target of multiple second messenger cascades, involving p38 MAPK, PKC, and PKA [96]. Mounir found that in the small and medium diameter neurons of the DRG,

CCL2 binding to the receptor CCR2 stimulates PLCβ by coupling to Gβγ dimers released by G proteins, which mediates activation of PKCε [84], a PKC isoform that enhances Nav1.8 currents and produces voltage-dependent changes in activation and inactivation [97,98]. Zhao et al. found that PKC also regulates Nav1.8 expression by promoting Nav1.8 phosphorylation and activating NF-κB, which is involved in ccl2-induced inflammatory hyperalgesia [94]. Not only that, but Baker also found that PKC is involved in TTX-R upregulation and sustained Na+ currents in sensory neurons of rats and mice [99]. And in one latest study, Abbadie proposed that Nav1.8 is a substrate for PKC [100], as well and attributed pain hypersensitivity to altered Nav1.8 function after phosphorylation by PKCε. In medium diameter DRG neurons, Wu et al. proved that CCL2/CCR2 signaling can modulate the function of its membrane surface Nav1.8 via the p38 MAPK pathway [101,102], which can be stimulated by dissociated βγ subunit stimulation [103]. In summary, chemokine CCL2 binds to receptor CCR2 to promote the expression of Nav1.8, which mediates nerve impulse conduction and induces inflammatory stimulation. Thus, the CCL2/CCR2 signaling upregulates Nav1.8 expression to increase nociceptors' excitability, further increasing the excitability of peripheral nociceptors in response to nerve injury (Figure 2).

The Role of CCL2\CCR2 in the Spinal Cord

Neuropathic pain is a manifestation of neuroplasticity that can manifest either as peripheral sensitization - increased sensitivity and excitability of primary sensory neurons in the Peripheral Nervous System (PNS), such as the increased excitability of DRG sensory neurons described above; or as central sensitization - -increased activity and excitability of nociceptive neurons in the spinal cord and brain in the Central Nervous System (CNS) and leads to the development and maintenance of neuropathic pain [104-106]. Nerve injury-induced central sensitization can be manifested as increasing excitatory synaptic transmission mediated by NMDA receptors and AMPA receptors in dorsal horn neurons, or as decreasing or losing inhibitory synaptic transmission mediated by GABA receptors and glycine receptors [107,108]. In addition to this, recently it is increasingly affirmed the relevance of glial cells (astrocytes and microglia) to neuropathic pain, as they form close interactions with neurons from which they can modulate nociceptive transmission in pathological conditions. Studies showing that nerve injury can trigger substantial changes in microglia and astrocytes in the spinal cord demonstrating the critical function of microglia and astrocytes in pain [109-111]. For example, Hua and Ledeboer et al. found that the microglia inhibitor minocycline prevented/retarded the progress of neuropathic pain by inhibiting microglia activation [112-114]; Jin et al. confirmed that neuropathic pain associated with astrocyte signaling pathways could be inhibited and attenuated by inhibiting JNK [115-117]. And in recent years, there is growing evidence by spinal cord glial cells can amplify and persist neuropathic pain through the release of pro-inflammatory cytokines, chemokines, and cytokines [118-121].

CCL2/CCR2 and Microglia

Microglia originate from the yolk sac and later develop into major immune cells (macrophages) in the central nervous system, regulating homeostasis in the brain and spinal cord [122]. Microglia development is mediated by the CSF1R and its ligand IL-34 [123,124]. In the CNS of healthy adults, microglia are present for life and maintained by their renewal, while circulating monocytes of bone marrow origin make little or no contribution [125,126].

Guan et al. found that microglia in the dorsal horn of the spinal cord are strongly activated in response to peripheral nerve injury [127,128], as manifested as a remarkable growth in number, as well as morphological changes, from branching to amoeboid shape, and an increase in cell volume. It was demonstrated that microglia needed activators to achieve activation, including Colony-Stimulating Factor 1 (CSF1), several chemokines, neuropeptide-1, and neuroproteases [127,129]. Here, we focus on the exploration of the chemokine CCL2 and its receptor CCR2, and although numerous researches show that they are expressed in spinal cord dorsal horn neurons and glial cells in both healthy rats and animal models of neuropathic pain [29,56], the source of CCL2 in the spinal cord parenchyma and the expression of CCR2 are still controversial of which further confirmation is needed.

Zhang et al. found by immunohistochemistry that CCL2 translocates from the DRG to the spinal cord [34,63], confirming the contention that CCL2 translocates from the DRG to the dorsal horn of the spinal cord. Dansereau also found that CCL2-containing vesicles are released via axoplasmic translocation to the dorsal horn of the spinal cord, acting on CCR2-expressing microglia [28]. However, Dansereau et al. found CCL2 can also be secreted from spinal cord dorsal horn explants prepared from naïve rats in a calcium-dependent way, demonstrating the fact that CCL2 is released from neuronal cells [34]. Jung et al. demonstrated in experiments with transgenic mice that CCL2 expression is restrictive to the damaged DRG [8], so the conjecture about the origin of CCL2 needs to be further confirmed.

CCR2 was demonstrated to be associated with microglia activation in neuropathic pain [30,31], but CCR2 expression within microglia in the dorsal horn of the spinal cord is not yet clearly demonstrated; instead, Gao et al. reported CCR2 expression in spinal cord neurons [6,56]. Since microglia originate from the myeloid lineage and share many of the same commonalities with monocytes/macrophages, there is a large literature indicating that cells expressing CCR2 are considered microglia, as well as CCR2 is characterized as a receptor in microglia which can lead to microglia responding to peripheral nerve injury [63,130]. In contrast, it is also evident that microglia do not express CCR2; Butovsky et al. did not detect the expression of CCR2 mRNA in microglia of adult mouse brain [131-134], and Zuurman et al. did not find the expression of CCR2 mRNA earlier in cultured microglia [135]. Thus further validation is needed for the co-localization of CCR2 in microglia, but most of the current literature still suggests that CCR2 is present in microglia, where CCL2 binding to CCR2 leads to microglia activation and proliferation, and that activated microglia produce many neuromodulators that rapidly modulate synaptic plasticity with what is considered as a trriger of the formation of pain after tissue and nerve injury [106,136].

So how does the activation of microglia by the CCL2/CCR2 signaling lead to pain? The p38 phosphorylation pathway in microglia was found to be a prevalent way following the activation of their cell surface receptors. p38 is a member of Mitogen-Activated Protein Kinases (MAPKs) [5,137-139], and Ji et al. found that activation of the p38 phosphorylation pathway can further result in the production and emission of multiple mediators in microglia to promote the progression of neuropathic pain, for instance, TNF-α, IL-1β IL-6, BDNF, PGE2, etc [5]: among them, it was shown that BDNFk can induce central sensitization by de-inhibition [107]; TNF-α increases excitatory synaptic transmission by enhancing the frequency of spontaneous excitatory

postsynaptic currents and the amplitude of currents induced by AMPA or NMDAs; while IL-1β strengthens both k excitatory and inhibitory synaptic transmission [107]. Taken together, this suggests that activated microglia can release bioactive mediators to promote pain signaling at the spinal cord level, leading to neuropathic pain. In addition, the extracellular signal-regulated kinases ERK1/2 and ERK5 were both demonstrated to implicate in the regulation of neuropathic pain [140,141]. Calvo et al. found phosphorylation of ERK1/2 and ERK5 following nerve injury in spinal microglia, though inhibition of the ERK1 / 2 pathway could reduce microglia proliferation as well as pain hypersensitivity in response to peripheral nerve injury [142].

CCL2/CCR2 and Astrocytes

Interestingly, Gao et al. reported that CCL2 is expressed both in microglia and spinal astrocytes with upregulation after nerve injury [6]. TNF-α is known to be a key promoter of the inflammatory cascade reaction t which is the basis for the progression of neuropathic pain following nerve injury [143-145]. Croitoru-Lamoury et al. found that TNF-α induces JNK-dependent expression and release of CCL2 in activated astrocytes in vitro [146-1448]. Not only that, but it was found that intrathecal injection of TNF-αinduced JNK-dependent increase in CCL2 upregulation in the spinal cord [6]. Secondly, Luo et al. found that chemokines released from astrocytes (e.g., CCL2 and IP-10), could further activate astrocytes to produce more CCL2 through a positive feedback loop [146,149]. Taken together, this suggests that besides microglia, astrocytes are also quite important for the maintenance of neuropathic pain [111,114,141,150].

Consequently, we can conclude that CCL2 is produced by the JNK pathway of astrocytes and released to the spinal cord parenchyma, acting on cells expressing CCR2 and being responsible for the progression of neuropathic pain. Gosselin also demonstrated CCR2 can be constitutively expressed in spinal cord dorsal horn neurons, in addition to in the microglia of the spinal cord [151]. Thus, CCL2 can perform the function of a signaling molecule between astrocytes and neurons and between glial cells which is responsible for central sensitization after nerve injury (Figure 3),

CCL2 and Neurons

In addition to DRG neurons, CCR2 is demonstrated to be presented in the spinal cord neurons of the superficial or the deep dorsal horn [31], and Gao et al. demonstrated CCR2 was upregulated in spinal cord neurons after peripheral nerve injury [31]. As for CCL2, expression of CCL2 is induced in spinal cord astrocytes after nerve injury and activate neurons by binding to CCR2 to increase excitatory synaptic transmission (astrocyte-to-neuron signaling), further activating the ERK pathway in neurons within minutes, which supports central sensitization and the development of neuropathic pain [152]. This suggests that CCL2 is released from the presynaptic membrane (astrocytes) to act on CCR2 (CCR2 on neurons) expressed on the postsynaptic membrane. Xie et al. found that CCL2 increased NMDA-induced currents only in CCR2-positive excitatory neurons that expressed VGLUT2 but not VIAAT, and not only that, CCL2 also promoted NMDA currents in SOM excitatory interneurons [153]. And in cultured spinal cord neurons, Gosselin et al. found that CCL2 inhibited GABAergic transmission [151], providing further evidence to support direct effects of CCL2 expressing on neurons in spinal cord.

Second, Ji et al. demonstrated that ERK activation in dorsal horn neurons is nociceptive-specific and contributes significantly to the induction of central sensitization [154], which can be used as a marker of central sensitization [155]. CCL2 has been shown to rapidly activate ERK in dorsal horn neurons, which later activates NMDA receptors to further regulate synaptic transmission, thus providing further support for the idea that CCL2 has a direct effect on spinal cord neurons and is involved in central sensitization [107].

In summary, in addition to activating microglia, CCL2 can also have direct, rapid effects on neurons through synaptic mechanisms - promoting central sensitization by inducing ERK activation and enhancing the excitability of dorsal horn neurons (Figure 3).

CCL2\CCR2 Involving in Pain by Affecting the Blood-Spinal Cord Barrier (BSCB)

The blood-spinal cord barrier is a physical barrier between the blood and the spinal cord parenchyma, separating the peripheral and central into two relatively independent systems that help protect and modulate the spinal cord parenchyma and spinal cord homeostasis. The basic components of the BSCB are capillary endothelial cells, basal lamina, pericytes, and astrocytic infarct endothelial cells, which were joined by tight junctions providing a crucial function in determining barrier permeability. Essential components of tight junctions include the plasma membrane eliments claudin and occludin, and the cytoplasmic protein component occluden -1 or ZO-1 [156]. Pan and Wang et al. found that peripheral nerve injury results in the release of TNFα from monocytes/macrophages, which regulates the breakdown of BSCB. There is also evidence of elevated TNFα expression in the spinal cord within hours after spinal cord compression, caused both by early BSCB opening and by release of infiltrating monocytes/macrophages [157,158].

The elevation of TNFα in the spinal cord has been shown to lead to a rise in BSCB permeability, which occurs as a result of TNF α-mediated reduction in the expression of ZO-1 and occludin. Bradykinin, a vasodilator mediated through NF-kβ activation, also indirectly regulates vascular permeability because of its effect on TNFα expression. Thus, the production of TNF α by monocytes/macrophages is induced following peripheral nerve injury, which mediates a decrease in the expression of the components of the BSCB, leading to altered permeability of the BSCB. The chemokine CCL2 can recruit monocytes/macrophages to the site of injury due to its induction properties, and thus CCL2 may act as an upstream signal in the monocyte-TNFα-alteration of the BSCB permeability pathway.

Currently, there is much evidence that the BSCB is disrupted in neuropathic pain models of nerve injury, so we speculate that the BSCB could act as a hub connecting the periphery and the center, causing interaction between the periphery and the center. However, many of the signaling molecular mechanisms have not been studied clearly, so the BSCB could be a potential target for new pain treatment. In conclusion, there are still many unknowns on the road to exploring neuropathic pain, and we believe that with the in-depth study of neuropathic pain mechanisms, this medical problem of neuropathic pain will be gradually solved for the ultimate benefit of many neuropathic pain patients.

Conclusion

The CCL2/CCR2 signaling pathway has been shown to act a critical function in the generation and maintenance of neuro-

pathic pain, and experimental evidence from various neuropathic pain models suggests that CCL2 is an appealing objective for intervention in neuropathic pain. However, to date, there is a large clinical gap in therapeutic approaches targeting interfering CCL2/CCR2 chemokine pairs. CCL2 is involved in pain transmission at all stages of the neurotransmission pathway and, in the periphery (both in damaged nerves and within the DRG), might work as a local autocrine\paracrine signal (neuronal signaling), stimulating second-order neurons in the pain cascade and/or attracting CCR2-expressing peripheral monocytes/macrophages. Also, CCL2 in the center (spinal paracrine) contributes to neuronal-microglia signaling and is a key for the upregulation of spinal microglia and astrocyte expression, a crucial step in the cascade response leading to neuropathic pain. Not only that, but central BSCB has also been proven to relate to neuropathic pain, and the pathway mediating pain production is contributed to CCL2. Exploring the mechanism by which CCL2 mediates BSCB-induced neuropathic pain needs further insight. Therefore, chemokine CCL2 has a significant function in the progression of neuropathic pain and is a potential drug target for the prevention of peripheral nerve injury forming neuropathic pain.

Author Statements

Acknowledgement

The work was supported by the Sichuan Provincial Department of Science and Technology (No.2021YJ0181), the Luzhou Bureau of Science, Technology and Talents ([2019] 151-6), and the Southwest Medical University (No.2020XYLH-053) (No.2021ZKQN122).

References

1. Yalcin I, Barthas F, Barrot M. Emotional consequences of neuropathic pain: insight from preclinical studies. Neurosci Biobehav Rev. 2014; 47: 154-64.

2. Szczudlik A, Dobrogowski J, Wordliczek J, Stępień A, Krajnik M, Leppert W, et al. Diagnosis and management of neuropathic pain: review of literature and recommendations of the Polish Association for the study of pain and the Polish Neurological Society - part one. Neurol Neurochir Pol. 2014; 48: 262-71.

3. Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. Nat Rev Drug Discov. 2014; 13: 533-48.

4. Gao YJ, Ji RR. Targeting astrocyte signaling for chronic pain. Neurotherapeutics. 2010; 7: 482-93.

5. Ji RR, Berta T, Nedergaard M. Glia and pain: is chronic pain a gliopathy? Pain. 2013; 154: S10-28-s28.

6. Gao YJ, Zhang L, Samad OA, Suter MR, Yasuhiko K, Xu ZZ, et al. JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain. J Neurosci. 2009; 29: 4096-108.

7. Zhang ZJ, Cao DL, Zhang X, Ji RR, Gao YJ. Chemokine contribution to neuropathic pain: respective induction of CXCL1 and CXCR2 in spinal cord astrocytes and neurons. Pain. 2013; 154: 2185-97.

8. Biber K, Boddeke E. Neuronal CC chemokines: the distinct roles of CCL21 and CCL2 in neuropathic pain. Front Cell Neurosci. 2014; 8: 210.

9. Jiang BC, Cao DL, Zhang X, Zhang ZJ, He LN, Li CH, et al. CXCL13 drives spinal astrocyte activation and neuropathic pain via CXCR5. J Clin Invest. 2016; 126: 745-61.

10. Clark AK, Malcangio M. Fractalkine/CX3CR1 signaling during neuropathic pain. Front Cell Neurosci. 2014; 8: 121.

11. Zhang ZJ, Dong YL, Lu Y, Cao S, Zhao ZQ, Gao YJ. Chemokine CCL2 and its receptor CCR2 in the medullary dorsal horn are involved in trigeminal neuropathic pain. J Neuroinflammation. 2012; 9: 136.

12. Gao YJ, Ji RR. Chemokines, neuronal-glial interactions, and central processing of neuropathic pain. Pharmacol Ther. 2010; 126: 56-68.

13. Ji RR, Woolf CJ. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. Neurobiol Dis. 2001; 8: 1-10.

14. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity. 2000; 12: 121-7.

15. Ji RR, Strichartz G. Cell signaling and the genesis of neuropathic pain. Sci STKE. 2004; 2004: reE14.

16. Yoshimura T, Robinson EA, Tanaka S, Appella E, Kuratsu J, Leonard EJ. Purification and amino acid analysis of two human glioma-derived monocyte chemoattractants. J Exp Med. 1989; 169: 1449-59.

17. Mélik-Parsadaniantz S, Rostène W. Chemokines and neuromodulation. J Neuroimmunol. 2008; 198: 62-8.

18. Semple BD, Kossmann T, Morganti-Kossmann MC. Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. J Cereb Blood Flow Metab. 2010; 30: 459-73.

19. White FA, Feldman P, Miller RJ. Chemokine signaling and the management of neuropathic pain. Mol Interv. 2009; 9: 188-95.

20. Zhang ZJ, Jiang BC, Gao YJ. Chemokines in neuron-glial cell interaction and pathogenesis of neuropathic pain. Cell Mol Life Sci. 2017; 74: 3275-91.

21. Savarin-Vuaillat C, Ransohoff RM. Chemokines and chemokine receptors in neurological disease: raise, retain, or reduce? Neurotherapeutics. 2007; 4: 590-601.

22. O'Connor T, Borsig L, Heikenwalder M. CCL2-CCR2 signaling in disease pathogenesis. Endocr Metab Immune Disord Drug Targets. 2015; 15: 105-18.

23. Lim SY, Yuzhalin AE, Gordon-Weeks AN, Muschel RJ. Targeting the CCL2-CCR2 signaling axis in cancer metastasis. Oncotarget. 2016; 7: 28697-710.

24. Ramesh G. Novel therapeutic targets in neuroinflammation and neuropathic pain. Inflamm Cell Signal. 2014; 1: e111.

25. Abbadie C, Bhangoo S, De Koninck Y, Malcangio M, Melik-Parsadaniantz S, White FA. Chemokines and pain mechanisms. Brain Res Rev. 2009; 60: 125-34.

26. Gosselin RD, Dansereau MA, Pohl M, Kitabgi P, Beaudet N, Sarret P, et al. Chemokine network in the nervous system: a new target for pain relief. Curr Med Chem. 2008; 15: 2866-75.

27. Tanaka T, Minami M, Nakagawa T, Satoh M. Enhanced production of monocyte chemoattractant protein-1 in the dorsal root ganglia in a rat model of neuropathic pain: possible involvement in the development of neuropathic pain. Neurosci Res. 2004; 48: 463-9.

28. Dansereau MA, Gosselin RD, Pohl M, Pommier B, Mechighel P, Mauborgne A, et al. Spinal CCL2 pronociceptive action is no longer effective in CCR2 receptor antagonist-treated rats. J Neurochem. 2008; 106: 757-69.

29. Thacker MA, Clark AK, Bishop T, Grist J, Yip PK, Moon LD, et al. CCL2 is a key mediator of microglia activation in neuropathic pain states. Eur J Pain. 2009; 13: 263-72.

30. Zhang J, Shi XQ, Echeverry S, Mogil JS, De Koninck Y, Rivest S. Expression of CCR2 in both resident and bone marrow-derived microglia plays a critical role in neuropathic pain. J Neurosci. 2007; 27: 12396-406.

31. Abbadie C, Lindia JA, Cumiskey AM, Peterson LB, Mudgett JS, Bayne EK, et al. Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. Proc Natl Acad Sci U S A. 2003; 100: 7947-52.

32. Knerlich-Lukoschus F, Juraschek M, Blömer U, Lucius R, Mehdorn HM, Held-Feindt J. Force-dependent development of neuropathic central pain and time-related CCL2/CCR2 expression after graded spinal cord contusion injuries of the rat. J Neurotrauma. 2008; 25: 427-48.

33. Bhangoo SK, Ripsch MS, Buchanan DJ, Miller RJ, White FA. Increased chemokine signaling in a model of HIV1-associated peripheral neuropathy. Mol Pain. 2009; 5: 48.

34. Van Steenwinckel J, Reaux-Le Goazigo A, Pommier B, Mauborgne A, Dansereau MA, Kitabgi P, et al. CCL2 released from neuronal synaptic vesicles in the spinal cord is a major mediator of local inflammation and pain after peripheral nerve injury. J Neurosci. 2011; 31: 5865-75.

35. Mennicken F, Maki R, de Souza EB, Quirion R. Chemokines and chemokine receptors in the CNS: a possible role in neuroinflammation and patterning. Trends Pharmacol Sci. 1999; 20: 73-8.

36. Miller RJ, Rostene W, Apartis E, Banisadr G, Biber K, Milligan ED, et al. Chemokine action in the nervous system. J Neurosci. 2008; 28: 11792-5.

37. Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. Nat Neurosci. 2007; 10: 1361-8.

38. White FA, Jung H, Miller RJ. Chemokines and the pathophysiology of neuropathic pain. Proc Natl Acad Sci U S A. 2007; 104: 20151-8.

39. Lindborg JA, Mack M, Zigmond RE. Neutrophils are critical for myelin removal in a peripheral nerve injury model of Wallerian degeneration. J Neurosci. 2017; 37: 10258-77.

40. Mildner A, Marinkovic G, Jung S. Murine monocytes: origins, subsets, fates, and functions. Microbiol Spectr. 2016; 4.

41. Mueller M, Leonhard C, Wacker K, Ringelstein EB, Okabe M, Hickey WF, et al. Macrophage response to peripheral nerve injury: the quantitative contribution of resident and hematogenous macrophages. Lab Invest. 2003; 83: 175-85.

42. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment [rep]. F1000Prime Rep. 2014; 6: 13.

43. Dubový P. Wallerian degeneration and peripheral nerve conditions for both axonal regeneration and neuropathic pain induction. Ann Anat. 2011; 193: 267-75.

44. Abbadie C, Bhangoo S, De Koninck Y, Malcangio M, Melik-Parsadaniantz S, White FA. Chemokines and pain mechanisms. Brain Res Rev. 2009; 60: 125-34.

45. Griffin JW, George R, Ho T. Macrophage systems in peripheral nerves. A review. J Neuropathol Exp Neurol. 1993; 52: 553-60.

46. Dubový P. Wallerian degeneration and peripheral nerve conditions for both axonal regeneration and neuropathic pain induction. Ann Anat. 2011; 193: 267-75.

47. Tofaris GK, Patterson PH, Jessen KR, Mirsky R. Denervated Schwann cells attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. J Neurosci. 2002; 22: 6696-703.

48. Mack M, Cihak J, Simonis C, Luckow B, Proudfoot AE, Plachý J, et al. Expression and characterization of the chemokine receptors CCR2 and CCR5 in mice. J Immunol. 2001; 166: 4697-704.

49. Brück W. The role of macrophages in Wallerian degeneration. Brain Pathol. 1997; 7: 741-52.

50. Krames ES. The role of the dorsal root ganglion in the development of neuropathic pain. Pain Med. 2014; 15: 1669-85.

51. Esposito MF, Malayil R, Hanes M, Deer T. Unique characteristics of the dorsal root ganglion as a target for neuromodulation. Pain Med. 2019; 20: S23-30-s30.

52. Xiao HS, Huang QH, Zhang FX, Bao L, Lu YJ, Guo C, et al. Identification of gene expression profile of dorsal root ganglion in the rat peripheral axotomy model of neuropathic pain. Proc Natl Acad Sci U S A. 2002; 99: 8360-5.

53. Liem L, van Dongen E, Huygen FJ, Staats P, Kramer J. The dorsal root ganglion as a therapeutic target for chronic pain. Reg Anesth Pain Med. 2016; 41: 511-9.

54. Harrison C, Epton S, Bojanic S, Green AL, FitzGerald JJ. The efficacy and safety of dorsal root ganglion stimulation as a treatment for neuropathic pain: A literature review. Neuromodulation. 2018; 21: 225-33.

55. White FA, Bhangoo SK, Miller RJ. Chemokines: integrators of pain and inflammation. Nat Rev Drug Discov. 2005; 4: 834-44.

56. White FA, Sun J, Waters SM, Ma C, Ren D, Ripsch M, et al. Excitatory monocyte chemoattractant protein-1 signaling is up-regulated in sensory neurons after chronic compression of the dorsal root ganglion. Proc Natl Acad Sci U S A. 2005; 102: 14092-7.

57. Sun JH, Yang B, Donnelly DF, Ma C, LaMotte RH. MCP-1 enhances excitability of nociceptive neurons in chronically compressed dorsal root ganglia. J Neurophysiol. 2006; 96: 2189-99.

58. Xie WR, Deng H, Li H, Bowen TL, Strong JA, Zhang JM. Robust increase of cutaneous sensitivity, cytokine production and sympathetic sprouting in rats with localized inflammatory irritation of the spinal ganglia. Neuroscience. 2006; 142: 809-22.

59. Subang MC, Richardson PM. Influence of injury and cytokines on synthesis of monocyte chemoattractant protein-1 mRNA in peripheral nervous tissue. Eur J Neurosci. 2001; 13: 521-8.

60. Taskinen HS, Röyttä M. Increased expression of chemokines (MCP-1, MIP-1alpha, RANTES) after peripheral nerve transection. J Peripher Nerv Syst. 2000; 5: 75-81.

61. Lindia JA, McGowan E, Jochnowitz N, Abbadie C. Induction of CX3CL1 expression in astrocytes and CX3CR1 in microglia in the spinal cord of a rat model of neuropathic pain. J Pain. 2005; 6: 434-8.

62. Milligan ED, Zapata V, Chacur M, Schoeniger D, Biedenkapp J, O'Connor KA, et al. Evidence that exogenous and endogenous fractalkine can induce spinal nociceptive facilitation in rats. Eur J Neurosci. 2004; 20: 2294-302.

63. Zhang J, De Koninck Y. Spatial and temporal relationship between monocyte chemoattractant protein-1 expression and spinal glial activation following peripheral nerve injury. J Neurochem. 2006; 97: 772-83.

64. Kleinschnitz C, Brinkhoff J, Sommer C, Stoll G. Contralateral cytokine gene induction after peripheral nerve lesions: dependence on the mode of injury and NMDA receptor signaling. Brain Res Mol Brain Res. 2005; 136: 23-8.

65. Hingtgen CM. A new "kine" of pain: MCP-1 and sensory neuron excitability. Focus on "MCP-1 enhances excitability of nociceptive neurons in chronically compressed dorsal root ganglia". J Neurophysiol. 2006; 96: 2171-2.

66. Vit JP, Ohara PT, Tien DA, Fike JR, Eikmeier L, Beitz A, et al. The analgesic effect of low dose focal irradiation in a mouse model of bone cancer is associated with spinal changes in neuro-mediators of nociception. Pain. 2006; 120: 188-201.

67. Khasabova IA, Stucky CL, Harding-Rose C, Eikmeier L, Beitz AJ, Coicou LG, et al. Chemical interactions between fibrosarcoma cancer cells and sensory neurons contribute to cancer pain. J Neurosci. 2007; 27: 10289-98.

68. Verge GM, Milligan ED, Maier SF, Watkins LR, Naeve GS, Foster AC. Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) distribution in spinal cord and dorsal root ganglia under basal and neuropathic pain conditions. Eur J Neurosci. 2004; 20: 1150-60.

69. Jung H, Miller RJ. Activation of the nuclear factor of activated T-cells (NFAT) mediates upregulation of CCR2 chemokine receptors in dorsal root ganglion (DRG) neurons: a possible mechanism for activity-dependent transcription in DRG neurons in association with neuropathic pain. Mol Cell Neurosci. 2008; 37: 170-7.

70. Watkins LR, Maier SF. Glia: a novel drug discovery target for clinical pain. Nat Rev Drug Discov. 2003; 2: 973-85.

71. Thacker MA, Clark AK, Marchand F, McMahon SB. Pathophysiology of peripheral neuropathic pain: immune cells and molecules. Anesth Analg. 2007; 105: 838-47.

72. Huang J, Zhang X, McNaughton PA. Modulation of temperature-sensitive TRP channels. Semin Cell Dev Biol. 2006; 17: 638-45.

73. Ouyang K, Zheng H, Qin X, Zhang C, Yang D, Wang X, et al. Ca2+ sparks and secretion in dorsal root ganglion neurons. Proc Natl Acad Sci U S A. 2005; 102: 12259-64.

74. Huang LY, Neher E. Ca(2+)-dependent exocytosis in the somata of dorsal root ganglion neurons. Neuron. 1996; 17: 135-45.

75. Jung H, Toth PT, White FA, Miller RJ. Monocyte chemoattractant protein-1 functions as a neuromodulator in dorsal root ganglia neurons. J Neurochem. 2008; 104: 254-63.

76. Qin X, Wan Y, Wang X. CCL2 and CXCL1 trigger calcitonin gene-related peptide release by exciting primary nociceptive neurons. J Neurosci Res. 2005; 82: 51-62.

77. Moore C, Gupta R, Jordt SE, Chen Y, Liedtke WB. Regulation of pain and itch by TRP channels. Neurosci Bull. 2018; 34: 120-42.

78. Oh SB, Tran PB, Gillard SE, Hurley RW, Hammond DL, Miller RJ. Chemokines and glycoprotein120 produce pain hypersensitivity by directly exciting primary nociceptive neurons. J Neurosci. 2001; 21: 5027-35.

79. Kao DJ, Li AH, Chen JC, Luo RS, Chen YL, Lu JC, et al. CC chemokine ligand 2 upregulates the current density and expression of TRPV1 channels and Nav1.8 sodium channels in dorsal root ganglion neurons. J Neuroinflammation. 2012; 9: 189.

80. Ruparel NB, Patwardhan AM, Akopian AN, Hargreaves KM. Homologous and heterologous desensitization of capsaicin and mustard oil responses utilize different cellular pathways in nociceptors. Pain. 2008; 135: 271-9.

81. Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, et al. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron. 2004; 41: 849-57.

82. Tominaga M, Caterina MJ. Thermosensation and pain. J Neurobiol. 2004; 61: 3-12.

83. Spicarova D, Adamek P, Kalynovska N, Mrozkova P, Palecek J. TRPV1 receptor inhibition decreases CCL2-induced hyperalgesia. Neuropharmacology. 2014; 81: 75-84.

84. Belkouch M, Dansereau MA, Réaux-Le Goazigo A, Van Steenwinckel J, Beaudet N, Chraibi A, et al. The chemokine CCL2 increases Nav1.8 sodium channel activity in primary sensory neurons through a Gβγ-dependent mechanism. J Neurosci. 2011; 31: 18381-90.

85. Bear B, Asgian J, Termin A, Zimmermann N. Small molecules targeting sodium and calcium channels for neuropathic pain. Curr Opin Drug Discov Devel. 2009; 12: 543-61.

86. Bhattacharya A, Wickenden AD, Chaplan SR. Sodium channel blockers for the treatment of neuropathic pain. Neurotherapeutics. 2009; 6: 663-78.

87. Matulenko MA, Scanio MJ, Kort ME. Voltage-gated sodium channel blockers for the treatment of chronic pain. Curr Top Med Chem. 2009; 9: 362-76.

88. Priest BT. Future potential and status of selective sodium channel blockers for the treatment of pain. Curr Opin Drug Discov Devel. 2009; 12: 682-92.

89. Dib-Hajj SD, Cummins TR, Black JA, Waxman SG. Sodium channels in normal and pathological pain. Annu Rev Neurosci. 2010; 33: 325-47.

90. Lampert A, O'Reilly AO, Reeh P, Leffler A. Sodium channelopathies and pain. Pflugers Arch. 2010; 460: 249-63.

91. Devor M. Sodium channels and mechanisms of neuropathic pain. J Pain. 2006; 7: S3-S12.

92. Cummins TR, Sheets PL, Waxman SG. The roles of sodium channels in nociception: implications for mechanisms of pain. Pain. 2007; 131: 243-57.

93. Dib-Hajj SD, Binshtok AM, Cummins TR, Jarvis MF, Samad T, Zimmermann K. Voltage-gated sodium channels in pain states: role in pathophysiology and targets for treatment. Brain Res Rev. 2009; 60: 65-83.

94. Zhao R, Pei GX, Cong R, Zhang H, Zang CW, Tian T. PKC-NF-κB are involved in CCL2-induced Nav1.8 expression and channel function in dorsal root ganglion neurons. Biosci Rep. 2014; 34.

95. Bajetto A, Bonavia R, Barbero S, Schettini G. Characterization of chemokines and their receptors in the central nervous system: physiopathological implications. J Neurochem. 2002; 82: 1311-29.

96. Dib-Hajj SD, Black JA, Waxman SG. Voltage-gated sodium channels: therapeutic targets for pain. Pain Med. 2009; 10: 1260-9.

97. Smrcka AV. G protein βγ subunits: central mediators of G protein-coupled receptor signaling. Cell Mol Life Sci. 2008; 65: 2191-214.

98. Cang CL, Zhang H, Zhang YQ, Zhao ZQ. PKCepsilon-dependent potentiation of TTX-resistant Nav1.8 current by neurokinin-1 receptor activation in rat dorsal root ganglion neurons. Mol Pain. 2009; 5: 33.

99. Baker MD. Protein kinase C mediates up-regulation of tetrodotoxin-resistant, persistent Na+ current in rat and mouse sensory neurones. J Physiol. 2005; 567: 851-67.

100. Wu DF, Chandra D, McMahon T, Wang D, Dadgar J, Kharazia VN, et al. PKCε phosphorylation of the sodium channel NaV1.8 increases channel function and produces mechanical hyperalgesia in mice. J Clin Invest. 2012; 122: 1306-15.

101. Jin X, Gereau RWt. Acute p38-mediated modulation of tetrodotoxin-resistant sodium channels in mouse sensory neurons by tumor necrosis factor-alpha. J Neurosci. 2006; 26: 246-55.

102. Hudmon A, Choi JS, Tyrrell L, Black JA, Rush AM, Waxman SG, et al. Phosphorylation of sodium channel Na(v)1.8 by p38 mitogen-activated protein kinase increases current density in dorsal root ganglion neurons. J Neurosci. 2008; 28: 3190-201.

103. Goldsmith ZG, Dhanasekaran DN. G protein regulation of MAPK networks. Oncogene. 2007; 26: 3122-42.

104. Ji RR, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci. 2003; 26: 696-705.

105. Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature. 2001; 413: 203-10.

106. Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science. 2000; 288: 1765-9.

107. Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, et al. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature. 2005; 438: 1017-21.

108. Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet. 1999; 353: 1959-64.

109. DeLeo JA, Tanga FY, Tawfik VL. Neuroimmune activation and neuroinflammation in chronic pain and opioid tolerance/hyperalgesia. Neuroscientist. 2004; 10: 40-52.

110. Jin SX, Zhuang ZY, Woolf CJ, Ji RR. p38 mitogen-activated protein kinase is activated after a spinal nerve ligation in spinal cord microglia and dorsal root ganglion neurons and contributes to the generation of neuropathic pain. J Neurosci. 2003; 23: 4017-22.

111. Zhuang ZY, Wen YR, Zhang DR, Borsello T, Bonny C, Strichartz GR, et al. A peptide c-Jun N-terminal kinase (JNK) inhibitor blocks mechanical allodynia after spinal nerve ligation: respective roles of JNK activation in primary sensory neurons and spinal astrocytes for neuropathic pain development and maintenance. J Neurosci. 2006; 26: 3551-60.

112. Hua XY, Svensson CI, Matsui T, Fitzsimmons B, Yaksh TL, Webb M. Intrathecal minocycline attenuates peripheral inflammation-induced hyperalgesia by inhibiting p38 MAPK in spinal microglia. Eur J Neurosci. 2005; 22: 2431-40.

113. Ledeboer A, Sloane EM, Milligan ED, Frank MG, Mahony JH, Maier SF, et al. Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation. Pain. 2005; 115: 71-83.

114. Raghavendra V, Tanga F, DeLeo JA. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. J Pharmacol Exp Ther. 2003; 306: 624-30.

115. Kawasaki Y, Xu ZZ, Wang X, Park JY, Zhuang ZY, Tan PH, et al. Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. Nat Med. 2008; 14: 331-6.

116. Romero-Sandoval EA, Horvath R, Landry RP, DeLeo JA. Cannabinoid receptor type 2 activation induces a microglial anti-inflammatory phenotype and reduces migration via MKP induction and ERK dephosphorylation. Mol Pain. 2009; 5: 25.

117. Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, et al. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature. 2003; 424: 778-83.

118. Abbadie C. Chemokines, chemokine receptors and pain. Trends Immunol. 2005; 26: 529-34.

119. Inoue K. The function of microglia through purinergic receptors: neuropathic pain and cytokine release. Pharmacol Ther. 2006; 109: 210-26.

120. Milligan ED, Watkins LR. Pathological and protective roles of glia in chronic pain. Nat Rev Neurosci. 2009; 10: 23-36.

121. Watkins LR, Maier SF. Beyond neurons: evidence that immune and glial cells contribute to pathological pain states. Physiol Rev. 2002; 82: 981-1011.

122. Crotti A, Ransohoff RM. Microglial physiology and pathophysiology: insights from genome-wide transcriptional profiling. Immunity. 2016; 44: 505-15.

123. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science. 2010; 330: 841-5.

124. Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, et al. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. Nat Immunol. 2012; 13: 753-60.

125. Tay TL, Mai D, Dautzenberg J, Fernández-Klett F, Lin G, Sagar, et al. A new fate mapping system reveals context-dependent random or clonal expansion of microglia. Nat Neurosci. 2017; 20: 793-803.

126. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci. 2007; 10: 1538-43.

127. Guan Z, Kuhn JA, Wang X, Colquitt B, Solorzano C, Vaman S, et al. Injured sensory neuron-derived CSF1 induces microglial proliferation and DAP12-dependent pain. Nat Neurosci. 2016; 19: 94-101.

128. Tsuda M, Inoue K, Salter MW. Neuropathic pain and spinal microglia: a big problem from molecules in ”small” glia. Trends Neurosci. 2005; 28: 101-7.

129. Okubo M, Yamanaka H, Kobayashi K, Dai Y, Kanda H, Yagi H, et al. Macrophage-colony stimulating factor derived from injured primary afferent induces proliferation of spinal microglia and neuropathic pain in rats. PLOS ONE. 2016; 11: e0153375.

130. Fernández-López D, Faustino J, Derugin N, Wendland M, Lizasoain I, Moro MA, et al. Reduced infarct size and accumulation of microglia in rats treated with WIN 55,212-2 after neonatal stroke. Neuroscience. 2012; 207: 307-15.

131. Beutner C, Linnartz-Gerlach B, Schmidt SV, Beyer M, Mallmann MR, Staratschek-Jox A, et al. Unique transcriptome signature of mouse microglia. Glia. 2013; 61: 1429-42.

132. Olah M, Amor S, Brouwer N, Vinet J, Eggen B, Biber K, et al. Identification of a microglia phenotype supportive of remyelination. Glia. 2012; 60: 306-21.

133. Hickman SE, Kingery ND, Ohsumi TK, Borowsky ML, Wang LC, Means TK, et al. The microglial sensome revealed by direct RNA sequencing. Nat Neurosci. 2013; 16: 1896-905.

134. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, et al. Identification of a unique TGF-β-dependent molecular and functional signature in microglia. Nat Neurosci. 2014; 17: 131-43.

135. Zuurman MW, Heeroma J, Brouwer N, Boddeke HW, Biber K. LPS-induced expression of a novel chemokine receptor (L-CCR) in mouse glial cells in vitro and in vivo. Glia. 2003; 41: 327-36.

136. Luo C, Kuner T, Kuner R. Synaptic plasticity in pathological pain. Trends Neurosci. 2014; 37: 343-55.

137. Clark AK, Staniland AA, Marchand F, Kaan TK, McMahon SB, Malcangio M. P2X7-dependent release of interleukin-1beta and nociception in the spinal cord following lipopolysaccharide. J Neurosci. 2010; 30: 573-82.

138. Kobayashi K, Yamanaka H, Fukuoka T, Dai Y, Obata K, Noguchi K. P2Y12 receptor upregulation in activated microglia is a gateway of p38 signaling and neuropathic pain. J Neurosci. 2008; 28: 2892-902.

139. Trang T, Beggs S, Wan X, Salter MW. P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. J Neurosci. 2009; 29: 3518-28.

140. Calvo M, Zhu N, Grist J, Ma Z, Loeb JA, Bennett DL. Following nerve injury neuregulin-1 drives microglial proliferation and neuropathic pain via the MEK/ERK pathway. Glia. 2011; 59: 554-68.

141. Zhuang ZY, Gerner P, Woolf CJ, Ji RR. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. Pain. 2005; 114: 149-59.

142. Tatsumi E, Yamanaka H, Kobayashi K, Yagi H, Sakagami M, Noguchi K. RhoA/ROCK pathway mediates p38 MAPK activation and morphological changes downstream of P2Y12/13 receptors in spinal microglia in neuropathic pain. Glia. 2015; 63: 216-28.

143. Sommer C, Lindenlaub T, Teuteberg P, Schäfers M, Hartung T, Toyka KV. Anti-TNF-neutralizing antibodies reduce pain-related behavior in two different mouse models of painful mononeuropathy. Brain Res. 2001; 913: 86-9.

144. Winkelstein BA, Rutkowski MD, Sweitzer SM, Pahl JL, DeLeo JA. Nerve injury proximal or distal to the DRG induces similar spinal glial activation and selective cytokine expression but differential behavioral responses to pharmacologic treatment. J Comp Neurol. 2001; 439: 127-39.

145. Schäfers M, Svensson CI, Sommer C, Sorkin LS. Tumor necrosis factor-alpha induces mechanical allodynia after spinal nerve ligation by activation of p38 MAPK in primary sensory neurons. J Neurosci. 2003; 23: 2517-21.

146. Croitoru-Lamoury J, Guillemin GJ, Boussin FD, Mognetti B, Gigout LI, Chéret A, et al. Expression of chemokines and their receptors in human and simian astrocytes: evidence for a central role of TNF alpha and IFN gamma in CXCR4 and CCR5 modulation. Glia. 2003; 41: 354-70.

147. Meeuwsen S, Persoon-Deen C, Bsibsi M, Ravid R, van Noort JM. Cytokine, chemokine and growth factor gene profiling of cultured human astrocytes after exposure to proinflammatory stimuli. Glia. 2003; 43: 243-53.

148. Mojsilovic-Petrovic J, Callaghan D, Cui H, Dean C, Stanimirovic DB, Zhang W. Hypoxia-inducible factor-1 (HIF-1) is involved in the regulation of hypoxia-stimulated expression of monocyte chemoattractant protein-1 (MCP-1/CCL2) and MCP-5 (CCL12) in astrocytes. J Neuroinflammation. 2007; 4: 12.

149. Luo Y, Fischer FR, Hancock WW, Dorf ME. Macrophage inflammatory protein-2 and KC induce chemokine production by mouse astrocytes. J Immunol. 2000; 165: 4015-23.

150. Wei F, Guo W, Zou S, Ren K, Dubner R. Supraspinal glial-neuronal interactions contribute to descending pain facilitation. J Neurosci. 2008; 28: 10482-95.

151. Gosselin RD, Varela C, Banisadr G, Mechighel P, Rostene W, Kitabgi P, et al. Constitutive expression of CCR2 chemokine receptor and inhibition by MCP-1/CCL2 of GABA-induced currents in spinal cord neurones. J Neurochem. 2005; 95: 1023-34.

152. Lyu Z, Guo Y, Gong Y, Fan W, Dou B, Li N, et al. The role of neuroglial crosstalk and synaptic plasticity-mediated central sensitization in acupuncture analgesia. Neural Plast. 2021; 2021: 8881557.

153. Xie RG, Gao YJ, Park CK, Lu N, Luo C, Wang WT, et al. Spinal CCL2 promotes central sensitization, long-term potentiation, and inflammatory pain via CCR2: further insights into molecular, synaptic, and cellular mechanisms. Neurosci Bull. 2018; 34: 13-21.

154. Ji RR, Baba H, Brenner GJ, Woolf CJ. Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. Nat Neurosci. 1999; 2: 1114-9.

155. Gao YJ, Ji RR. c-Fos and pERK, which is a better marker for neuronal activation and central sensitization after noxious stimulation and tissue injury? Open Pain J. 2009; 2: 11-7.

156. Ryu JK, Petersen MA, Murray SG, Baeten KM, Meyer-Franke A, Chan JP, et al. Blood coagulation protein fibrinogen promotes autoimmunity and demyelination via chemokine release and antigen presentation. Nat Commun. 2015; 6: 8164.

157. Pan W, Kastin AJ, Bell RL, Olson RD. Upregulation of tumor necrosis factor alpha transport across the blood-brain barrier after acute compressive spinal cord injury. J Neurosci. 1999; 19: 3649-55.

158. Wang CX, Nuttin B, Heremans H, Dom R, Gybels J. Production of tumor necrosis factor in spinal cord following traumatic injury in rats. J Neuroimmunol. 1996; 69: 151-6.