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REVIEW

Chemokines in neuron–glial cell interaction and pathogenesis of neuropathic pain

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Abstract Neuropathic pain resulting from damage or dysfunction of the nervous system is a highly debilitating chronic pain state and is often resistant to currently available treatments. It has become clear that neuroinflammation, mainly mediated by proinflammatory cytokines and chemokines, plays an important role in the establishment and maintenance of neuropathic pain. Chemokines were originally identified as regulators of peripheral immune cell trafficking and were also expressed in neurons and glial cells in the central nervous system. In recent years, accumulating studies have revealed the expression, distribution and function of chemokines in the spinal cord under chronic pain conditions. In this review, we provide evidence showing that several chemokines are upregulated after peripheral nerve injury and contribute to the pathogenesis of neuropathic pain via different forms of neuron–glia interaction in the spinal cord. First, chemokine CX3CL1 is expressed in primary afferents and spinal neurons and induces microglial activation via its microglial receptor CX3CR1 (neuron-to-microglia signaling). Second, CCL2 and CXCL1 are expressed in spinal astrocytes and act on CCR2 and CXCR2 in spinal neurons to increase excitatory synaptic transmission (astrocyte-to-neuron signaling). Third, we recently identified that CXCL13 is highly upregulated in spinal neurons after spinal nerve ligation and induces spinal astrocyte activation via receptor CXCR5 (neuron-to-astrocyte signaling). Strategies that target chemokine-mediated neuron-glia interactions may lead to novel therapies for the treatment of neuropathic pain.

Keywords Neuroinflammation · Astrocytes · Microglia · Spinal cord · Chronic pain

Abbreviations

AD	Alzheimer's disease
AMPA	Alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
BCP	Bone cancer pain
CCI	Chronic constriction injury
CFA	Complete Freund's adjuvant
CNS	Central nervous system
CSF	Cerebrospinal fluid
DRG	Dorsal root ganglion
EAE	Experimental autoimmune encephalomyelitis
ERK	Extracellular signal-regulated kinase
EPSC	Excitatory postsynaptic currents
GABA	Gamma-aminobutyric acid
IL	Interleukin
IAMNT	Inferior alveolar nerve and mental nerve transection
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MCP-1	Monocytes chemoattractant protein-1
MS	Multiple sclerosis
LTP	Long-term potentiation
NMDA	*N*-methyl-D-aspartic acid
pIONL	Partial infraorbital nerve ligation
PNS	Peripheral nervous system
pSNL	Partial sciatic nerve ligation

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SNL	Spinal nerve ligation
SNI	Spared nerve injury
TNF-α	Tumor necrosis factor-alpha
TG	Trigeminal ganglion

Introduction

Neuropathic pain, resulting from a direct consequence of various lesions or diseases affecting the somatosensory nervous system, is common clinically but there is a lack of effective treatments [1]. Neuropathic pain is manifested as spontaneous pain, hyperalgesia (enhanced pain evoked by a noxious stimulus), and allodynia (pain evoked by innocuous stimulus) [1]. It has been thought that neural plasticity, which includes both peripheral sensitization in the dorsal root ganglion (DRG), and central sensitization in the spinal cord and supraspinal areas, is responsible for the development and maintenance of neuropathic pain [2–4]. Based on this concept, neuronal mechanisms underlying neuropathic pain have been widely investigated. Indeed, after nerve injury, a variety of receptors, transmitters, and inflammatory mediators are upregulated in DRG neurons, which increase the neuronal excitability and contribute to peripheral sensitization [2, 5–7]. In the spinal cord, nerve injury increases NMDA receptor- and AMPA receptor-mediated excitatory synaptic transmission, and decreases GABA receptor- and glycine receptor-mediated inhibitory synaptic transmission in dorsal horn neurons which contribute to central sensitization [8–10].

In recent years, it has been accepted that besides neurons, non-neuronal cells such as immune cells (macrophages and lymphocytes) and glial cells [Schwann cells and satellite cells in the peripheral nervous system (PNS), and astrocytes and microglia in the central nervous system (CNS)] play an important role in the induction and maintenance of neuropathic pain [11–15]. Nerve injury induces sequential activation of microglia and astrocytes in the spinal cord. Microglia marker (CD11b) mRNA is increased at 4 h post nerve injury, continued to increase at day 14, and returned to basal levels at day 28 [16]. In contrast, astrocyte marker (GFAP) mRNA is increased from day 4 post nerve injury, maintained at high level at day 28 [16]. Inhibition of microglial activation by minocycline prevents or delays the development of neuropathic pain [12, 17, 18], whereas inhibition of astrocytes by fluorocitrate or L-α-aminoadipate alleviates established neuropathic pain [11, 17]. Given that both astrocytes and microglia have physiological functions and inhibition of glial activation by toxins may cause side effects [19], a deep understanding of the communication between glial cells and neurons under chronic pain conditions is essential for the development of novel therapies.

Neuroinflammation has been well demonstrated to play an important role in the pathogenesis of neurodegenerative diseases and chronic pain [4]. Glial cells contribute to neuroinflammation by releasing proinflammatory cytokines [such as the tumor necrosis factor (TNF-α) and interleukin-1β (IL-1β)], growth factors, and chemokines (such as CCL2 and CXCL1) [4, 11, 20–22]. On the other hand, inflammatory mediators are also released from primary afferents and spinal neurons to induce glial activation [4, 23–25]. Among the inflammatory mediators, chemokines are implicated in peripheral sensitization and central sensitization after nerve injury or tissue damage [4, 21, 22, 24–26]. In this review, we summarize the evidence for the involvement of chemokines and chemokine receptors in neuropathic pain, focusing on how chemokines regulate nerve injury-induced neuropathic pain via different forms neuron–glia interaction in the spinal cord.

Chemokines and receptors

Chemokines are small secreted proteins (8–14 kDa) that were initially characterized as chemotactic peptides to control the trafficking of leukocytes [6, 27, 28]. Chemokines have conserved cysteine residues in their amino acid sequences. Based on the presence and position of the first cysteine residues, chemokines are classified into four subfamilies: CC, CXC, XC, and CX3C [2]. The CC group has the first two of total four cysteines in adjacent position, the CXC group has the two of the four cysteines separated by an intervening animo acid, the XC group has only two cysteine residues, and the CX3C group has three amino acids between two cysteine residues. Approximately 50 chemokines have been identified in mammalian species. The CC group and CXC group are the largest groups containing 28 (CCL1-28) and 16 (CXCL1-16) members, respectively. The XC group includes two members (XCL1 and XCL2), and the CX3C group has only one member CX3CL1.

The functions of chemokines are exerted through their respective receptors, which are seven-transmembrane-domain G-protein-coupled receptors (GPCRs) with a serine/threonine-rich intracellular C-terminal domain and an acidic N-terminal extracellular domain. Chemokine receptors are divided into different families, CC chemokine receptors (CCR1-10), CXC chemokine receptors (CXCR1-7), XC chemokine receptors (XCR1), and CX3C chemokine receptors (CX3CR1), that correspond to the four distinct subfamilies of chemokines they bind. Except for some monogamous chemokine–chemokine receptor pairs, such as CXCL13–CXCR5, CXCL16–CXCR6, and CX3CL1–CX3CR1, most chemokines activate multiple receptors. A single receptor can also be activated by diverse

chemokines [2, 29]. The large number of chemokines and the promiscuous interaction of chemokines and receptors make their functions complex.

It is well known that chemokines play an important role in the immune system. Studies show that chemokines are also involved in several other processes, including cardiogenesis, vascular development, cell proliferation, angiogenesis, and metastasis [30–33]. In particular, chemokines and their receptors are involved in the pathogenesis of neurodegenerative diseases such as multiple sclerosis (MS), Alzheimer's disease (AD), as well as in neurological disorders, such as stroke and trauma [34–36]. In recent years, the expression, distribution, and function of chemokines and chemokine receptors in neuropathic pain have been investigated in different animal models, such as spinal nerve ligation (SNL) [37], spared nerve injury (SNI) [38], chronic constriction of sciatic nerve (CCI) [39], partial sciatic nerve ligation (pSNL) [40], and chronic compression of the DRG (CCD) [41]. Chemokine pairs CX3CL1/CX3CR1 has been demonstrated to be involved in neuropathic pain via neuron–microglia interaction in the spinal cord (see reviews [2, 25, 27, 29, 42]). Recent studies showed that CXCL1/CXCR2 and CXCL13/CXCR5 contribute to neuropathic pain via different forms of neuron-glia interaction.

Chemokines contribute to the pathogenesis of neuropathic pain via different forms of neuron–glia interaction in the spinal cord

CX3CL1–CX3CR1 regulates neuropathic pain via neuron–microglia interaction

CX3CL1 (also called fractalkine), the only member of the CX3C group, is constitutively expressed in the normal PNS and CNS, where it is found in neurons [43, 44]. This profile was also confirmed by the CX3CL1-mCherry transgenic mice [45]. However, CX3CL1 can be induced in spinal astrocytes by peripheral nerve injury [46]. CX3CL1 is a unique chemokine in that it binds to only one known receptor (CX3CR1), and that chemokine receptor binds only CX3CL1 [47, 48].

The expression and distribution of CX3CL1 and CX3CR1 after nerve injury

CX3CL1 is also unique as it has two forms ($a \sim 100$ kDa large form and $a \sim 80$ kDa small form) corresponding to membrane-bound and soluble form, respectively. Neuronal injury or peripheral nerve injury does not change the total expression of CX3CL1 mRNA in brain and spinal neurons, but the membrane-associated CX3CL1 protein is decreased [25, 43, 44, 49]. However, a recent report showed that CX3CL1 protein is upregulated in spinal neurons via NFκB-dependent H4 acetylation in paclitaxel-induced neuropathic pain [50]. Peripheral nerve injury also induces a marked reduction of membrane-bound form and an increase of soluble form in the DRG [51]. Dorsal rhizotomy greatly reduces CX3CL1 immunoreactivity in nerve fibers in the spinal dorsal horn, suggesting that DRG neurons are also the source of the CX3CL1 in the spinal dorsal horn [43]. The shedding of mature CX3CL1 is executed by various proteases, including cathepsin S (CatS), metalloproteases, ADAM10 (a closely related disintegrin-like metalloproteinase 10), and ADAM-17 (TACE, tumor necrosis factor-alpha converting enzyme) [25, 52–54]. CatS is expressed in vascular smooth cells and can cleave CX3CL1 into a soluble smaller size form (~55 kDa) [55]. In vitro study shows that CatS incubation reduces the level of sensory neuron-associated CX3CL1 on the neuron membrane [56]. In the spinal cord, CatS is secreted by activated spinal microglia and upregulated after nerve injury to cleave the soluble CX3CL1 from spinal local neurons and primary afferent fibers [49, 56]. The metalloproteases also play an important role in shedding of mature CX3CL1. Application of the general matrix metalloprotease inhibitor batimastat dose-dependently inhibits the cleavage of CX3CL1 from the membranes of glutamate-treated primary cortical neurons [44]. Whether ADAM-17 or ADAM-10 contributes to soluble CX3CL1 shedding in the spinal cord has not been evaluated.

CX3CR1 is found on the microglia of CNS, satellite glial cells of DRG, and macrophages of peripheral nerves, but not in astrocytes, neurons, or oligodendrocytes [25, 57, 58]. In naïve or CFA (Complete Freund's adjuvant)-injected animals, the immunoreactivity of CX3CR1 in spinal microglia is weak [46]. However, following peripheral nerve injury or bone cancer, the extensive upregulation of CX3CR1 occurs in microglia of the dorsal horn [43, 46, 51, 58].

CX3CL1–CX3CR1 mediates neuron–microglia interaction in the spinal cord

Currently, the mechanisms underlying CX3CL1-induced pain facilitation are well demonstrated. In peripheral nerve, the CX3CR1-expressing macrophages are critical for the initial development of chemotherapy-induced neuropathic pain [59]. In the DRG, CX3CR1-expressing satellites contribute to the genesis of inflammatory pain [60]. In the spinal cord, the CatS from activated microglia results in the production of soluble CX3CL1, which acts on CX3CR1 of microglia [49, 61]. Therefore, CatS-CX3CL1–CX3CR1 forms a positive feedback loop in peripheral nerve injury-induced neuropathic pain [49, 56, 61].

P38, a member of mitogen-activated protein kinases (MAPKs), is an important downstream kinase of CX3CL1/CX3CR1 signaling [51, 56]. Intrathecal delivery of a CX3CR1 neutralizing antibody decreases the activation of p38 in spinal microglia following SNL [51]. Conversely, intrathecal injection of CX3CL1 induces p38 activation in microglia [51, 56]. Activation of p38 induces synthesis of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 in microglia [62], which can regulate synaptic transmission in the superficial spinal dorsal horn [2]. Accumulating evidence has shown that activated microglia contribute to spinal long-term potentiation (LTP) and pathological pain [63, 64]. CX3CL1/CX3CR1 signaling is also involved in tetanic stimulation of the sciatic nerve-induced pain hypersensitivity and LTP in the spinal dorsal horn [65, 66].

CX3CL1 and CX3CR1 contribute to neuropathic pain

A growing body of evidence supports the involvement of CX3CL1 in neuropathic pain. Intrathecal administration of CX3CL1 induces dose-dependent mechanical allodynia and thermal hyperalgesia in naive rats and mice [56, 67]. In addition, intrathecal administration of CX3CL1 neutralizing antibody reverses neuropathic pain and CatS-induced mechanical hyperalgesia [56]. Intrathecal delivery of CX3CR1-neutralizing antibody attenuates the development of neuropathic pain and also blocks CX3CL1-induced mechanical allodynia and thermal hyperalgesia in naïve animals [60, 67]. In *Cx3cr1* knockout (KO) mice, microglial activation is reduced in the spinal cord, mechanical allodynia is not developed, and thermal hypersensitivity is delayed following pSNL compared to their C57BL/6 background wild type (WT) littermates [58]. However, it was also reported that *Cx3cr1* KO mice display an increase in allodynia for 3 weeks after SNI compared to strain-matched Balb/c controls. In addition, intra-neural injection into the sciatic nerve of CX3CL1 delayed the development of alldynia for 3 days following SNI [68]. The reason for this discrepancy is not clear, but it could be due to the differences in the mice (C57Bl/6 vs. Balb/c) and the models (pSNL vs. SNI). In addition, as most of chemokines and receptors are widely expressed in different cell types in the PNS and CNS, cre-lox systems are needed in future studies to clarify the specific role of a chemokine/receptor in different cell type and different anatomical area.

CCL2–CCR2 and CXCL1–CXCR2 regulate neuropathic pain via astrocyte–neuron interaction in the spinal cord

CCL2–CCR2

CCL2 is also known as monocyte chemoattractant protein 1 (MCP-1). CCL2 can activate CCR2 and CCR11. However, CCR2 is its preferred receptor [69–71]. Besides CCL2, CCR2 can also be activated by CCL7, CCL8, and CCL13. Binding analysis indicates that CCR2 binds CCL2 with ten times higher affinity than CCL7 and CCL8 in mouse tissues [72]. A recent study showed that CCL7 (monocyte chemotactic protein 3, MCP-3), which is produced by spinal astrocytes, acts on CCR2 in microglia to induce spinal microglial activation [73], suggesting that CCR2 can also be recognized by CCL7 in the spinal cord.

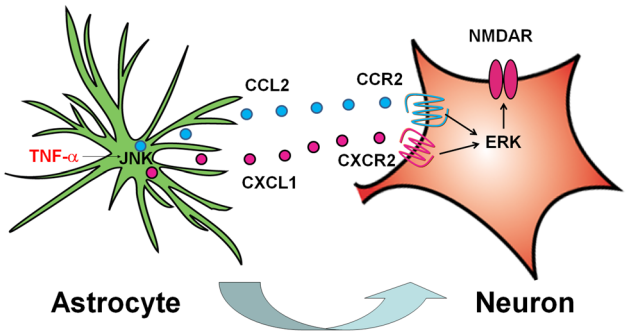

Fig. 1 Schematic shows CCL2/CCR2 and CXCL1/CXCR2 mediate astrocyte-to-neuron crosstalk in the spinal cord. SNL induces rapid TNF-α expression and release, which activates JNK and further induces CCL2 and CXCL1 expression in astrocytes. These chemokines are released from astrocytes to act on spinal neurons via CCR2 and CXCR2, respectively. The activated CCR2 and CXCR2 activates ERK, which may phosphorylate NMDA receptor and enhance synaptic transmission

The expression and distribution of CCL2 and CCR2 after nerve injury CCL2 is constitutively expressed in DRG neurons [74]. After peripheral nerve injury, CCL2 expression in ipsilateral DRG is dramatically upregulated in small and large neurons [75–78]. In addition, CCL2 in DRG neurons can also be transported to central terminal in the spinal cord. CCL2 is found in SP (Substance P)- and CGRP (Calcitonin rene-related peptide)-positive primary afferents in the superficial dorsal horn [21, 23, 70, 74, 79–81]. In vivo immunohistochemistry shows that CCL2 is also expressed in astrocytes of the spinal cord and is upregulated after nerve injury [21]. The increase of CCL2 expression in astrocytes is shown at days 3 and 10, and peaked at day 3 in the spinal cord after SNL [21]. In cultured astrocytes, TNF-α induces a c-Jun N-terminal kinase (JNK)-dependent expression and release of CCL2 [21]. Intrathecal injection of TNF-α also induces JNK-dependent CCL2 upregulation in the spinal cord [21] (Fig. 1). CCL2 is also expressed in astrocytes in the medulla oblongata after the inferior alveolar and mental nerve transection (IAMNT) or experimental tooth movement (ETM) [26, 82], as well as in brain after demyelinating injury [83], focal cerebral ischemia [84], and entorhinodentate lesions [85].

CCR2 is expressed and upregulated in the DRG after sciatic nerve demyelination [70, 76, 77]. Chronic constriction of DRG (CCD) induces CCR2 mRNA expression in neurons and non-neuronal cells in DRG [77]. In addition, CCR2 is partially colocalized with CCL2, suggesting a possible autocrine/paracrine role for CCL2/CCR2 signaling within the DRG [70]. In the spinal cord, CCR2 has low expression under normal conditions [21] and is upregulated in spinal microglia after peripheral nerve injury [23, 73, 79, 86]. However, our in situ hybridization data show that CCR2 is expressed and increased in spinal neurons after peripheral nerve injury [21]. Jung et al. also reported a weak but clear GFP signal in spinal dorsal horn neurons in CCR2-GFP reporter mice [76]. CCR2 is also upregulated in neurons of the ipsilateral medulla oblongata after IAMNT [26].

CCL2–CCR2 mediates astrocyte–neuron interaction in the spinal cord Previous studies showed that intrathecal injection of high-dose CCL2 induces microglial activation and mechanical allodynia in WT mice, but not in *Ccr2* KO mice [21, 87, 88]. In addition, microglial activation is not developed after peripheral nerve injury in *Ccr2* KO mice [79, 86]. Nerve injury-induced p38 activation in microglia is also attenuated in *Ccr2* KO mice [79, 86]. These data suggest that CCL2 is involved in microglial activation via CCR2 in the spinal cord under neuropathic pain condition.

However, accumulating evidence also supports that CCL2 and CCR2 mediate astrocyte–neuron interaction in the spinal cord. Electrophysiological studies show that CCL2 can immediately (within 2 min) enhance NMDA- and AMPA-induced current and increase the frequency of sEPSCs in spinal cord lamina II neurons [21]. Perfusion of spinal cord slices with CCL2 induces rapid phosphorylation of extracellular-signal-regulated kinase (ERK), a nociceptive-specific marker [89, 90] in superficial dorsal horn neurons within 5 min [21]. In addition, co-stimulation of CCL2 with GABA to cultured spinal cord neurons results in rapid dose-dependent reduction of GABA-induced inward currents [91]. These data suggest that CCL2, released from primary afferents and astrocytes, can act on CCR2 in neurons and regulate synaptic transmission in the spinal cord (Fig. 1).

CCL2 and CCR2 contribute to neuropathic pain Behavioral evidence shows that intrathecal administration of CCL2 induces rapid heat hyperalgesia, starting at 15 min and recovering at 24 h [21]. Mice over-expressing CCL2 in astrocytes display enhanced nociceptive responses [92]. Intrathecal injection of CCL2 neutralizing antibody reduces SNL- or CCI-induced mechanical allodynia [21, 88]. The siRNA targeting *Ccr2* reverses the nociceptive behaviors induced by CCL2 intrathecal injection or CFA intraplantar injection [93]. In addition, the development of mechanical allodynia is totally abrogated after peripheral nerve injury in *Ccr2* KO mice [79, 86]. The magnitude of the nocifensive behavior induced by IAMNT or ETM is reduced by intracisternal injection of CCR2 antagonists in a dose-dependent manner [26, 82].

CXCL1–CXCR2

CXCL1 is one member of the CXC family and also known as keratinocyte-derived chemokines (KC), growth-related oncogene (GRO), or cytokine-induced neutrophil chemoattractant-1 (CINC-1). CXCL1 in rodents has similar biological functions as interleukin-8 (IL-8) in human [94]. In the peripheral tissue, CXCL1 is expressed and secreted by activated macrophages, endothelial cells, and fibroblast cells. CXCL1 is involved in neutrophil chemotaxis and degranulation at the early stage of inflammation and is involved in inflammatory pain and acute post-incisional pain [95, 96]. The role of CXCL1 is dependent on its primary receptor CXCR2 [95, 96]. In brain tissue, CXCL1 is expressed by neurons, microglia, and oligodendrocytes [97–99]. CXCR2 has been detected on neurons, microglia, and oligodendrocyte progenitors in the brain [97–99].

The expression and distribution of CXCL1 and CXCR2 after nerve injury In the spinal cord, CXCL1 is weakly expressed in naïve animal and increased after peripheral nerve injury [22, 100]. ELISA shows that CXCL1 expression is increased from day 3, peaked at day 10, and maintained at day 21 after SNL. The upregulation of TNF-α is earlier than the increase of CXCL1 after SNL, and TNF-α inhibitor blocks SNL-induced CXCL1 upregulation [22], suggesting that TNF-α is an important trigger for CXCL1 expression in the spinal cord. In addition, immunostaining shows that CXCL1 is primarily expressed by astrocytes in naive mice and increased after SNL (Fig. 2a–c), spinal cord injury, or inoculation of cancer cells into the bone [22, 101, 102]. CXCL1 is induced in human astrocytes in the active multiple sclerosis lesions [103]. In cultured astrocytes, TNF-α increases the expression of connexin 43 (Cx43), a hemichannel expressed on astrocytes, and controls the release of astrocytic mediators. TNF-α-induced release of CXCL1 is blocked by carbenoxolone (a non-selective hemichannel blocker), Gap26/Gap27 (selective Cx43 blockers), or Cx43 siRNA [104], suggesting that CXCL1 is expressed in astrocytes and the release is controlled by gap junction.

CXCR2 mRNA and protein are also upregulated in the spinal cord in peripheral nerve injury-induced neuropathic pain, incisional postoperative pain, CFA-induced inflammatory pain, and bone cancer pain (BCP) [22, 102, 105, 106]. Immunostaining shows the CXCR2 is located

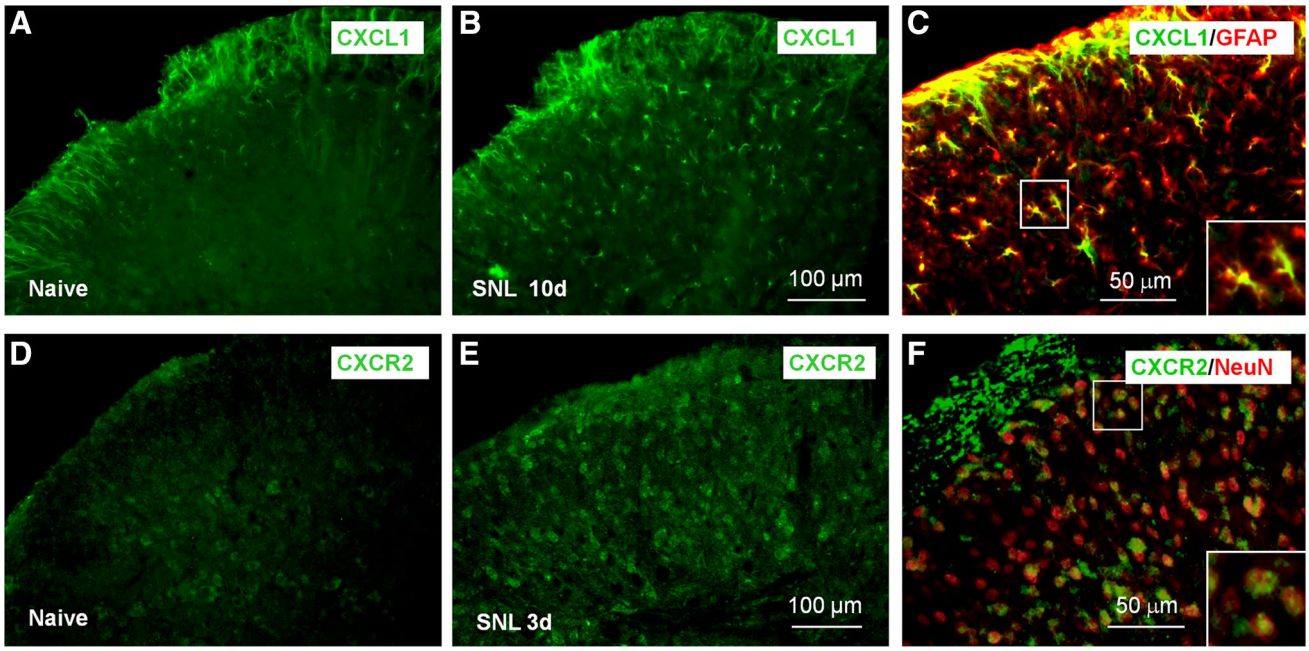

Fig. 2 SNL induces the upregulation of CXCL1 and CXCR2 in the spinal cord. **a, b** Immunostaining shows the expression of CXCL1 in the spinal cord of naïve mice (**a**) and SNL 10d mice (**b**). **c** Double staining shows that CXCL1 is colocalized with astrocytic marker GFAP in the spinal dorsal horn. **d, e** Immunostaining shows the expression of CXCR2 in the spinal cord of naïve mice (**d**) and SNL 3d mice (**e**). **f** Double staining shows that CXCR2 is colocalized with neuronal marker NeuN in the spinal dorsal horn. The images are reproduced from a paper by Zhang et al. [22] (*Pain*) with permission

in the spinal neurons (Fig. 2d–f), but not in spinal astrocytes or microglia in mice 10 days after SNL or 14 days after cancer cells inoculation into the bone [22, 102].

CXCL1–CXCR2 mediates astrocyte–neuron interaction in the spinal cord CXCL1 and CXCR2 are, respectively, expressed in astrocytes and neurons in the spinal cord, indicating that they may be involved in the interaction of astrocytes and neurons (Fig. 1). Indeed, intrathecal injection of CXCL1 not only induces CXCR2-dependent thermal hyperalgesia, but also induces ERK activation and c-Fos expression in spinal neurons [22]. Electrophysiological recording shows that CXCL1 increases NMDA-induced currents via CXCR2 in neurons of dorsal horn lamina II [22, 105]. Furthermore, nerve injury-induced sEPSC in spinal lamina IIo nociceptive synapses in the late phase is suppressed by carbenoxolone and Gap27 and recapitulated by CXCL1 [104]. Compared to the rapid (1 day) onset of neuropathic pain after SNL, CXCL1 is increased slowly (3 days) [22], indicating that CXCL1 may not initiate the neuropathic pain, but involves in the maintenance of neuropathic pain.

CXCL1 and CXCR2 contribute to neuropathic pain Inhibition of CXCL1 alleviates neuropathic pain induced by peripheral nerve injury. Intrathecal injection of CXCL1 neutralizing antibody partially reduces pSNL- and SNL-induced mechanical allodynia [22, 100]. Intraspinal injection of CXCL1 shRNA lentivirus vectors leads to persistent knockdown of CXCL1 expression, produces a marked and persistent anti-hyperalgesic and anti-allodynic effect in SNL mice [22], indicating the role of CXCL1 in the maintenance of neuropathic pain.

Intrathecal injection of CXCR2 antagonist SB225002 blocks CXCL1-induced heat hyperalgesia [22]. Intrathecal injection of SB225002 also dose-dependently reduces mechanical allodynia and heat hyperalgesia induced by SNL or hind paw incision [22, 107]. Intraperitoneal injection of SB225002 displays prominent and long-lasting antinociceptive effect in pSNL mice [108]. These data indicate that CXCL1/CXCR2 signaling is important in the pathogenesis of neuropathic pain.

Interestingly, CXCL1 level is increased in the cerebrospinal fluid (CSF) samples from opioid-tolerant cancer patients [109]. Rats with morphine tolerance also show increased CXCL1 mRNA in the spinal cord. In addition, the development of tolerance of morphine in rats is accelerated by coadministration of CXCL1 and attenuated by coadministration of CXCL1-neutralizing antibody or CXCR2 antagonist [109], suggesting that CXCL1/CXCR2 signaling may be a novel target for the treatment of opioid tolerance.

CXCL13–CXCR5 contributes to neuropathic pain via neuron–astrocyte interaction

CXCL13, also known as B lymphocyte chemoattractant (BLC), was originally identified to be produced by stromal cells in B cell follicles [110, 111]. CXCR5, the only receptor for CXCL13 [110] and also called CD185 (cluster of differentiation 185) or Burkitt lymphoma receptor 1 (BLR1), is expressed on all B cells and a subset of T cells in blood, lymphatic tissue, and CSF [112, 113]. CXCL13 strongly attracts B lymphocytes and some T cells and macrophages via CXCR5 in peripheral lymphoid organs such as spleen, lymph nodes, and Peyer's patches [114]. CXCL13 was thought not to be expressed in the healthy CNS but upregulated due to the infiltration of immune cells under pathological conditions [115–117]. For example, CXCL13 was found in infiltrating dendritic cells in the inflamed brain meninges and spinal cord in mice with experimental autoimmune encephalomyelitis (EAE) [115, 116]. Several studies showed that CXCL13 level correlates directly with the number of B cells in brain tissue and CSF [113], while successful treatment of multiple sclerosis patients is associated with parallel declines of CXCL13 levels and CSF B cell counts [118–120]. However, it was also reported that CXCL13-deficient animals show normal CNS B cell recruitment but mild, self-limited form of EAE [115, 121], indicating that CXCL13/CXCR5 in the CNS may regulate this neuroinflammatory disease via B cell-independent mechanisms.

The expression and distribution of CXCL13 and CXCR5 after nerve injury

A recent study showed that spinal cord has low level of CXCL13 and CXCR5 in naïve mice and non-diseased humans [24]. In addition, CXCL13 is the most upregulated gene in 37 detectable chemokines in the spinal cord 10 days after SNL. The 47-fold increase of CXCL13 is more than that of CCL7, CCL2, and CXCL1, which are known to be involved in neuropathic pain [21, 22, 73]. CXCL13 mRNA expression is increased as early as 1 day after SNL and persists for more than 21 days [24]. The protein level of this chemokine is also elevated in the ipsilateral dorsal horn of spinal cord (Fig. 3a, b), as well as in the CSF 10 days after SNL. Furthermore, CXCL13 is restricted to neurons (Fig. 3c), with no expression in astrocytes or microglia in the spinal cord [24]. Interestingly, the increase in CXCL13 gene transcription after SNL is regulated by a non-coding microRNA, miR-186-5p, which is downregulated after SNL via NMDA receptor in spinal neurons (Fig. 4) [122]. Besides the spinal cord, CXCL13 mRNA is increased in the DRG 14 days after local inflammation of DRG or 3 days after SNL or modified SNL model in rats [123]. CXCL13 mRNA and protein expression is also increased in

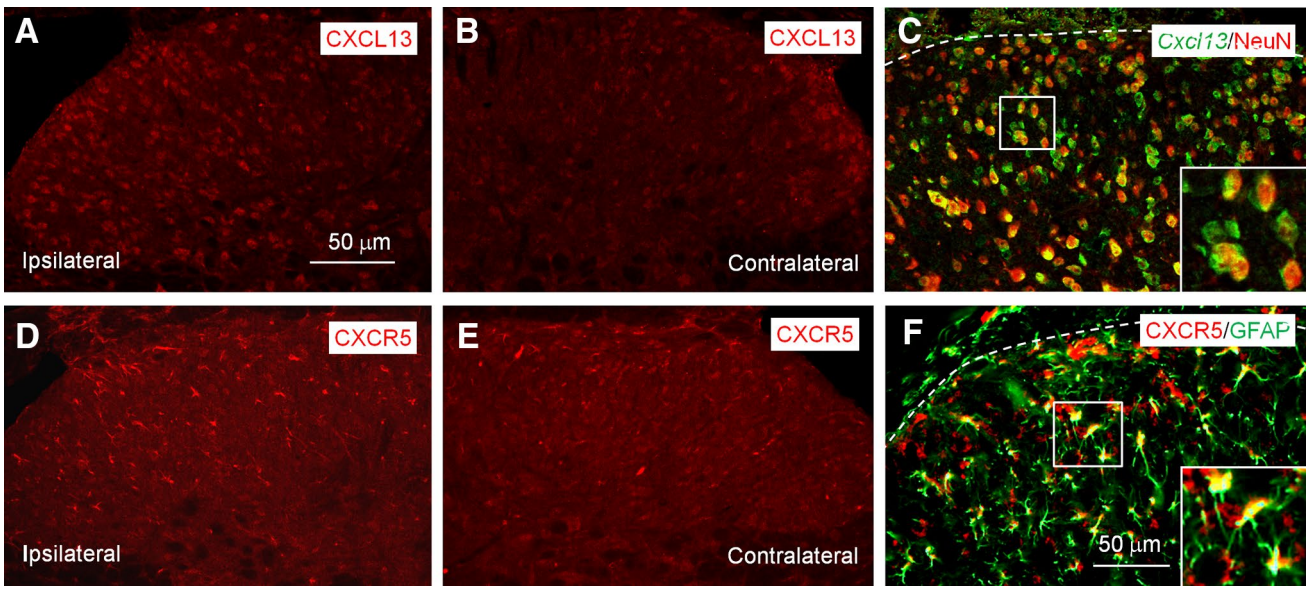

Fig. 3 SNL induces CXCL13 and CXCR5 expression in spinal neurons and astrocytes, respectively. **a, b** SNL increases CXCL13 expression in the ipsilateral spinal dorsal horn (**a**), but not in the contralateral spinal dorsal horn (**b**). **c** Double staining shows that CXCL13 is colocalized with NeuN in the spinal cord dorsal horn. **d, e** SNL increases CXCR5 expression in the ipsilateral spinal dorsal horn (**d**), but not in the contralateral spinal dorsal horn (**e**). **f** Double staining shows CXCR5 is colocalized with GFAP in the spinal cord. The images are re-produced from a paper by Jiang et al. [24] (*The Journal of Clinical Investigation*) with permission

the trigeminal ganglion (TG) after partial infraorbital nerve ligation (pIONL) [122].

The sole receptor of CXCL13, CXCR5 has low expression in naïve spinal cord but is increased in the ipsilateral dorsal horn after SNL (Fig. 3d, e), which is started from 1 day and maintained for more than 21 days. Interestingly, CXCR5 is mainly localized in astrocytes (Fig. 3f), with a few in neurons in the superficial dorsal horn [24]. Conversely, CXCR5 is expressed in neurons of TG and increased after pIONL [122]. These data suggest that CXCL13/CXCR5 may mediate pain processing via different mechanisms in the CNS and PNS.

CXCL13–CXCR5 mediates neuron–astrocyte interaction in the spinal cord

Due to the respective expression of CXCL13 and CXCR5 in spinal neurons and astrocytes, they may contribute to neuropathic pain via neuron–astrocyte interaction (Fig. 4). Indeed, astrocyte activation in the spinal cord was remarkably reduced in *Cxcr5*-deficient mice (Fig. 5a–d). Intrathecal injection of CXCL13 increases GFAP expression in the spinal cord 6 h after injection. Intrathecal CXCL13-induced pain hypersensitivity is inhibited by astrocyte functional inhibitor, L-α-aminoadipate [24]. Furthermore, CXCL13 induces CXCR5-dependent ERK activation in spinal astrocytes. SNL-induced ERK activation is reduced in the spinal cord in *Cxcr5*-deficient mice [24]. Importantly, CXCL13 induces ERK activation in cultured astrocytes. In addition, intrathecal injection of CXCL13-activated astrocytes from WT mice, but not from *Cxcr5*-deficient mice, induces mechanical allodynia in naive mice [24]. These data suggest that CXCL13 may activate astrocytes via CXCR5.

Interestingly, although CXCR5 is not expressed in spinal microglia, SNL-induced microglial activation is reduced in *Cxcr5*-deficient mice (Fig. 5e–h). Moreover, spinal injection of CXCL13 not only induces rapid astroglial activation at 6 h, but also induces delayed microglial activation at 24 h (Fig. 5i). Inhibition of either astrocyte function or microglia function inhibits microglia marker IBA-1 expression (Fig. 5j). These results suggest that activated astrocytes may further activate microglia in the spinal cord [24].

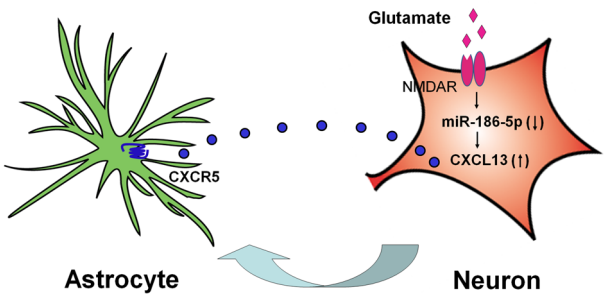

Fig. 4 Schematic shows CXCL13/CXCR5 mediates neuron-to-astrocyte crosstalk in the spinal cord. SNL increases glutamate release from presynaptic terminals to activate NMDAR on postsynaptic neurons, leading to downregulation of miR-186-5p, and this decrease results in CXCL13 upregulation in spinal neurons. Upon release, CXCL13 acts on CXCR5 in astrocytes to induce astrocytic activation

CXCL13 and CXCR5 contribute to neuropathic pain

Behavioral studies show that intraspinal injection of CXCL13 shRNA persistently attenuated SNL-induced mechanical allodynia. In addition, overexpression of spinal miR-186-5p decreases CXCL13 expression and attenuates SNL-induced pain hypersensitivity [24]. In contrast, inhibition of miR-186-5p is sufficient to increase CXCL13 expression and induce pain hypersensitivity. Knockdown of CXCR5 in the spinal cord by shRNA lentivirus also reverses SNL-induced pain hypersensitivity in WT mice for more than 4 weeks. Furthermore, the second injection of CXCR5 shRNA is still effective in reversing late-phase neuropathic pain [24]. Consistently, *Cxcr5* KO mice show marked deficits in SNL-induced heat hyperalgesia and mechanical allodynia [24]. In addition, inhibition of CXCL13/CXCR5 in the TG before or after pIONL attenuates pIONL-induced mechanical allodynia [122]. These data suggest that CXCL13/CXCR5 is necessary for the maintenance of neuropathic pain. Moreover, intrathecal injection of CXCL13 induces CXCR5-dependent heat hyperalgesia and mechanical allodynia [24]. Intra-TG injection of CXCL13 also induces orofacial pain [122], indicating that activation of CXCR5 is also sufficient to induce pain hypersensitivity. It is noteworthy that different from the cellular distribution of CXCL13 and CXCR5 in the spinal cord, CXCL13 and CXCR5 are both expressed in neurons of the trigeminal ganglion. Intra-TG injection of CXCL13 induces CXCR5-dependent ERK and pp38 activation and proinflammatory cytokine production [122, 124], suggesting a different mechanism of CXCL13/CXCR5 in the TG in mediating neuropathic pain.

Other chemokines that involve in neuropathic pain

Besides the chemokines discussed above, some other chemokiens are also involved in neuropathic pain.

CXCL10 (IFN-γ-inducible protein 10) is one of highly upregulated chemokines in the spinal cord after SNL [24]. It mainly recognizes CXCR3, which is also recognized by CXCL9 and CXCL11 [35]. Our recent study shows that CXCL10 is constitutively expressed in spinal neurons in naive mice, but increased in neurons and astrocytes after SNL [125]. SNL or cancer cells inoculation also increases CXCR3 expression in spinal neurons [125, 126]. *Cxcr3*-deficient mice show reduced mechanical allodynia and

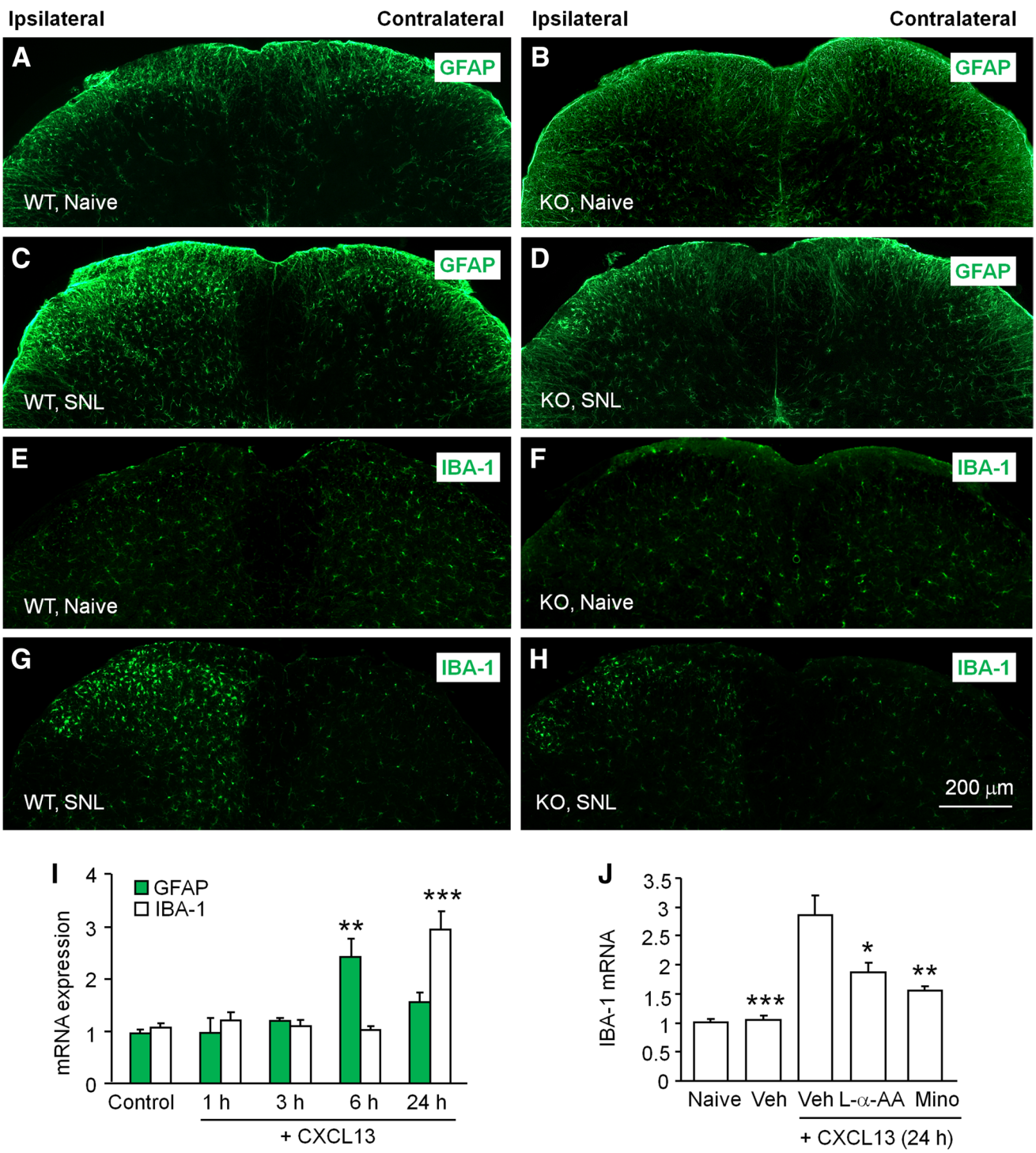

Fig. 5 SNL-induced activation of glial cells in the spinal cord is inhibited in CXCR5 KO mice. **a**–**d** GFAP staining in the spinal cord of naive WT and *Cxcr5* KO mice or mice after SNL (7 days). **e**–**h** IBA-1 staining in the spinal cord in naive WT and *Cxcr5* KO mice or mice after SNL (7 days). *Scale bar* 200 μm. **i** Intrathecal injection of CXCL13 increased GFAP mRNA at 6 h and IBA-1 mRNA at 24 h. $^{**}P<0.01$, $^{***}P<0.001$, vs. control. One-way ANOVA followed by Bonferroni's test. **j** Intrathecal injection of astroglial function inhibitor L-α-aminoadipate (L-α-AA) or microglial inhibitor minocycline (Mino) decreased CXCL13-induced IBA-1 mRNA upregulation in the spinal cord 24 h after CXCL13 injection. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, compared to Vehicle (Veh) + CXCL13. One-way ANOVA followed by Bonferroni's test. The images are re-produced from a paper by Jiang et al. [24] (*The Journal of Clinical Investigation*) with permission

heat hyperalgesia after SNL [125]. In addition, intrathecal injection of CXCR3 antagonist attenuates both neuropathic pain and bone cancer pain [125, 126]. Although it has been demonstrated that CXCL10 can induce pain via CXCR3 [125], the role of CXCL9 and CXCL11 has not been identified yet.

CXCL12 (stromal cell-derived factor 1, SDF-1), exerts function through its primary CXCR4 receptor. In the spinal cord, CXCL12 and CXCR4 are mainly colocalized with spinal neurons and also in spinal astrocytes and microglia [127–131]. Intrathecal injection of a CXCL12 neutralizing antibody or CXCR4 antagonist AMD3100 can delay or reverse the initiation and persistence of bone cancer pain and nerve injury-induced neuropathic pain [127, 129, 130, 132, 133].

CCL21, also named secondary lymphoid-tissue chemokine (SLC), is specifically located in large dense-core vesicles in damaged DRG neurons and transported into axons and pre-synaptic terminals [134]. CCR7, as the receptor of CCL21, can be induced in activated microglia in EAE animals [135]. The *Ccl21*-deficient mice do not develop neuropathic pain and fail to upregulate the P2X4 expression in activated microglia after peripheral nerve injury [136].

CCL3, also named macrophage inflammatory protein-1 alpha (MIP-1α), is found to participate in the development of neuropathic pain through its dominant receptors CCR1 and CCR5 [137]. In spinal dorsal horn, the distribution of CCL3 is still unclear. In the spinal dorsal horn, CCL3 and its receptors CCR1, but not CCR5 are increased after pSNL [138]. Intrathecal injection of CCL3 neutralizing antibody can prevent the mechanical allodynia and thermal hyperalgesia induced by pSNL [138, 139]. *Ccr5* KO mice do not develop mechanical allodynia and thermal hyperalgesia, and the spinal microglial reaction to the peripheral nerve injury is also impaired [139]. Considering there is no expression of CCR5 in the spinal cord, the analgesia effects in *Ccr5* KO mice may be due to the inhibition of inflammation that occurred in injured peripheral nerve.

Conclusion

It is clear that neuropathic pain states are associated with a profound neuroinflammation, in which chemokines play an important role in the development and maintenance of neuropathic pain. By the different distribution in neurons and glial cells of the ligand and receptor, chemokines can either induce glial activation or facilitate excitatory

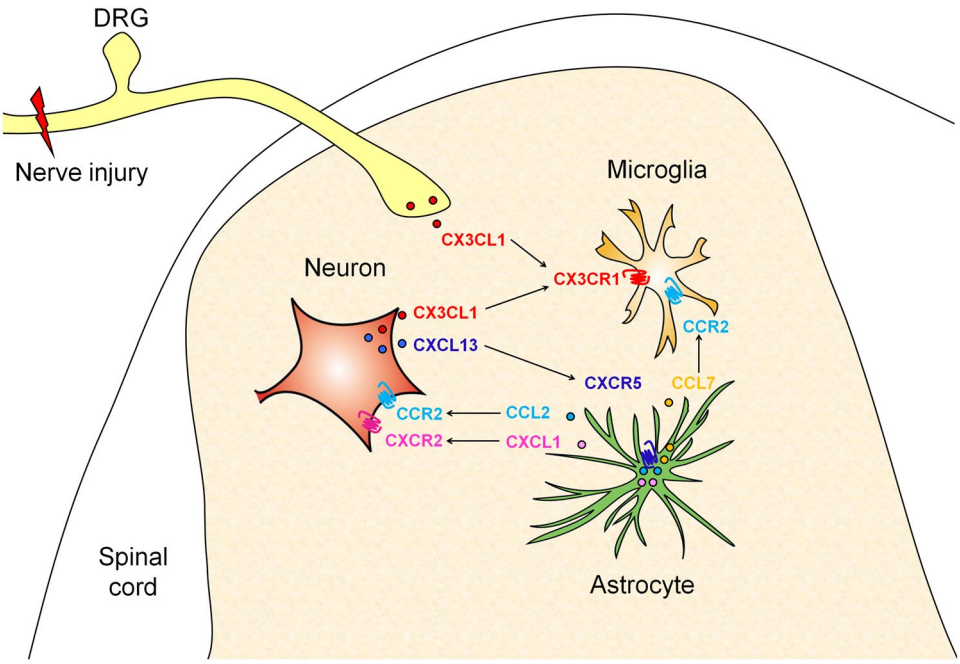

Fig. 6 A schematic shows the interactions between several chemokines and their receptors in the spinal cord in the neuropathic pain condition. After nerve injury, CX3CL1 is shed from primary afferents and spinal neurons and acts on its receptor CX3CR1 to induce microglia activation. CXCL13 releases from spinal neurons and acts on CXCR5 to induce astrocyte activation. The activated astrocytes release CCL2 and CXCL1, which act on their major receptor CCR2 and CXCR2 on spinal neurons to enhance excitatory synaptic transmission. CCL7 may be released from astrocytes and acts on CCR2 on microglia to activate microglia. Thus, microglia, astrocytes, neurons constitute a positive feedback loop via chemokine and receptor interactions to contribute to the pathogenesis of neuropathic pain

Table 1 The expression, distribution, and function of chemokine/receptor in the spinal cord under neuropathic pain condition

Pain model	Chemokine/receptor	Expression	Distribution	Phenotype of receptor KO mice	Pharmacological intervention to pain behavior (i.t.)	Neuronal-glial interaction	Ref.
CCI SNI SNL pSNL	CX3CL1	CX3CL1 Soluble form POD (1–7) (↑)	Primary afferents Neuron Astrocyte	Normal basal pain threshold pSNL-Mechanical allodynia (↓) pSNL-Heat hyperalgesia (↓) SNI-Mechanical allodynia (↑)	CX3CL1 neutralizing antibody (↓) CX3CR1neutralizing antibody (↓)	Neuron–microglia interaction	43, 46 51, 56 58, 61 68
	CX3CR1	POD (1–10) (↑)	Microglia				
CCI SNL IAMNT ETM	CCL2	POD (3–21) (↑)	Primary afferents Astrocyte	Normal basal pain threshold pSNL-Mechanical allodynia (↓)	CCL2 neutralizing antibody (↓) CCR2 siRNA (↓) CCR2 antagonist (INCB344, RS504393) (↓)	Neuron–astrocyte interaction	21, 78 79, 82 85, 86 88
	CCR2	POD (3–21) (↑)	Microglia Neuron				
SNL BCP	CXCL1	POD (3–21) (↑)	Astrocyte	Unknown	CXCL1 neutralizing antibody (↓) CXCL1 shRNA (↓) CXCR2 antagonist (SB225002) (↓)	Astrocyte–neuron interaction	22, 100 102, 104
	CXCR2	POD (3–21) (↑)	Neuron				
SNL	CXCL13	POD (1–21) (↑)	Neuron	Normal basal pain threshold SNL-Mechanical allodynia (↓) SNL-Heat hyperalgesia (↓)	CXCL13 shRNA (↓) CXCR5 shRNA (↓)	Neuron–astrocyte interaction	24
	CXCR5	POD (3–21) (↑)	Astrocyte				
SNL BCP	CXCL10	POD (3–21) (↑)	Neuron Astrocyte	Normal basal pain threshold SNL-Mechanical allodynia (↓) SNL-Heat hyperalgesia (↓)	CXCR3 shRNA (↓) CXCR3 antagonist (NBI-74330) (↓)	Astrocyte–neuron interaction	24, 125 126
	CXCR3	POD (3–21) (↑)	Neuron				
pSNL SNL SNI BCP	CXCL12	POD (3–21) (↑)	Neuron Astrocyte Microglia	Unknown	CXCL12 neutralizing antibody (↓) CXCR4 antagonist (AMD3100, AMD3465) (↓)	Unknown	127, 129 130, 132 133
	CXCR4	POD (5–21) (↑)	Neuron Astrocyte Microglia				
SNL	CCL21	POD (1–2) (↑)	Primary afferents	Normal basal pain threshold SNL-Mechanical allodynia (↓)	CCL21 neutralizing antibody (↓)	Unknown	136
	CCR7	POD (?)	Microglia				
pSNL SNL	CCL3	POD (3–14) (↑)	Unknown	Normal basal pain threshold SNL-Mechanical allodynia (↓) SNL-Heat hyperalgesia (↓)	CCL3 neutralizing antibody (↓)	Unknown	138, 139
	CCR1,CCR5	POD (3–7) (↑)	Unknown				

CCI chronic constriction injury, *IAMNT* inferior alveolar and mental nerve transection, *pSNL* partial sciatic nerve ligation, *SNL* spinal nerve ligation, *SNI* spared nerve injury, *BCP* bone cancer pain, *ETM* experimental tooth movement, *POD* post-operative days, *i.t.* intrathecal injection

synaptic transmission of spinal neurons, which exaggerates central sensitization (Fig. 6). Moreover, evidence supports that nerve injury induced a rapid microglial activation and a delayed and persistent astrocytic activation. Consistently, chemokines, such as CX3CL1, which induces microglia activation, are upregulated in the first few days after nerve injury, whereas CCL2, CXCL1, and CXCL10, which induce astrocyte activation, are upregulated after 3 days, and maintained for more than 21 days (Table 1). These findings suggest that targeting chemokine receptors, such as CX3CR1 after nerve injury may prevent the development of neuropathic pain. In contrast, blocking the chemokine receptors, such as CCR2, CXCR2, CXCR3, and CXCR5, may alleviate the established late phase neuropathic pain.

As we summarized in Table 1, siRNA or neutralizing antibody was used in most of the studies to block the function of chemokine signaling, and only a few chemokine receptor antagonists were tested. In recent years, pharmaceutical companies have developed a number of chemokine receptor antagonists (including antagonists for CCR1, CCR2, CCR3, CCR4, CCR5, CCR8, CCR9, CXCR1/CXCR2, CXCR2, CXCR3, and CXCR4) for the treatment of autoimmune-related or inflammation-related diseases, such as asthma, atopic diseases, AIDS, rheumatoid arthritis, and diabetes [140–142]. However, only two chemokine receptor antagonists have been licensed for clinical use: CCR5 inhibitor, Mavaviroc and CXCR4 inhibitor, Plerixafor. They show efficacy in the inhibition of HIV-1 entry (Miraviroc) and stem cell mobilization (Plerixafor) [143, 144]. Most of the chemokine receptor antagonists have not been tested for analgesic efficacy clinically. A CCR2 antagonist, AZD2423 (AstraZeneca), was tested for the treatment of posttraumatic neuralgia. Different from the anti-hyperalgesia effect on animals with neuropathic pain, it did not show efficacy on NRS (numerical rating scale) average pain scores of patients [145]. The reasons for the failures may attribute to species selectivity, the redundancy of the drug target, pharmacokinetic properties, and drug metabolism [143]. In addition, most of the preclinical studies checked the analgesic effect of drugs on stimulus-evoked pain, but NRS was evaluated in human. Thus, it is important to evaluate the efficacy of chemokine receptor antagonists on animals' spontaneous pain in the future. Given the important role of spinal chemokines and receptors in neuropathic pain and the redundancy in the chemokine network, development of receptor antagonists that have CNS permeability and can target several chemokine receptors may be beneficial for the treatment of neuropathic pain.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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