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Chemokines and pain mechanisms

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Abstract

The development of new therapeutic approaches to the treatment of painful neuropathies requires a better understanding of the mechanisms that underlie the development of these chronic pain syndromes. It is now well established that astrocytic and microglial cells modulate the neuronal mechanisms of chronic pain in spinal cord and possibly in the brain. In animal models of neuropathic pain following peripheral nerve injury, several changes occur at the level of the first pain synapse between the central terminals of sensory neurons and second order neurons. These neuronal mechanisms can be modulated by pronociceptive mediators released by non neuronal cells such as microglia and astrocytes which become activated in the spinal cord following PNS injury. However, the signals that mediate the spread of nociceptive signaling from neurons to glial cells in the dorsal horn remain to be established. Herein we provide evidence for two emerging signaling pathways between injured sensory neurons and spinal microglia: chemotactic cytokine ligand 2 (CCL2)/CCR2 and cathepsin S/CX3CL1 (fractalkine)/CX3CR1. We discuss the plasticity of these two chemokine systems at the level of the dorsal root ganglia and spinal cord demonstrating that modulation of chemokines using selective antagonists decrease nociceptive behavior in rodent chronic pain models. Since up-regulation of chemokines and their receptors may be a mechanism that directly and/or indirectly contributes to the development and maintenance of chronic pain, these molecular molecules may represent novel targets for therapeutic intervention in sustained pain states.

Keywords

Cathepsin S; CCR2; CX3CR1; Fractalkine; MCP-1; Neuropathy; Pain

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1. Introduction

Neuropathic pain is experienced in association with many types of injury to the nervous system or as a consequence of diabetes, cancer or infectious agents. From the therapeutic point of view, neuropathic pain often proves refractory to existing therapies. A better understanding of the cellular and molecular processes involved in the development of neuropathic pain is essential for the development of novel therapies. Many pathophysiological mechanisms underlie the development of neuropathic pain states. The site of these mechanisms include not only the damaged nerve and dorsal root ganglia (DRG), but also changes in the central processing of sensory information, most notably at the level of the spinal cord. This phenomenon has traditionally been considered a neuronally mediated response. Recent findings have highlighted the active involvement of glial cells in the pathogenesis of nerve injury-induced neuropathic pain and uncover new targets for potential analgesics (Watkins and Maier, 2003, Marchand et al., 2005, Tsuda et al., 2005, Scholz and Woolf, 2007). Microglia release a variety of mediators including pro-inflammatory cytokines and chemokines that contribute to pain signaling. Chemokines are small proteins that were initially characterized as chemotactic peptides controlling the trafficking of leukocytes (for review see Charo and Ransohoff, 2006). Chemokine classification is based on the presence and position of the first cysteine residues. The CC group has two adjacent cysteines, the CXC group has one amino acid that separates the two cysteine residues, and the CX3X (three amino acids between two cysteines residues) chemokine CX3CL1 (also termed fractalkine) has only one member in its class. Chemokines are released locally from peripheral blood cells at sites of inflammation and are crucial during the inflammatory response since they are accountable for leukocyte recruitment to the site of damage. In addition, chemokines are also involved in pain processing as described in this chapter.

1.1. Role of CCL2 in spinal nociceptive transmission

The understanding of the functional role of CCL2 in pain processing requires a precise knowledge of its distribution, and its co-localization with pain-related neuropeptides in DRG neurons and in their nerve terminals in the dorsal horn of the spinal cord. Within DRG, CCL2 is constitutively expressed in small and medium diameter size neurons under naïve conditions and in several chronic pain models (Fig. 1; Tanaka et al., 2004,White et al., 2005,Zhang and De Koninck, 2006). To identify the phenotype of CCL2-expressing cells, double-labeling immunohistochemistry combining CCL2 antibodies with classical neuronal markers revealed that CCL2-immunoreactivity was restricted to the cell bodies of DRG neurons. Among these, CCL2 labeling was detected in the subpopulation of peptidergic neurons immunoreactive for substance P and in primary sensory neurons expressing the neuropeptide CGRP (Dansereau et al., 2008). Moreover, CCL2-positive neurons also co-localized extensively with the capsaicin-heat and proton-gated ion channel, transient receptor potential vanilloid 1 (Dansereau et al., 2008). At higher magnification, confocal images of dually stained DRG neurons revealed that the intracellular pattern of staining for CCL2 only partially overlapped with those of substance P or CGRP (Fig. 1). Indeed, the neuropeptides substance P and CGRP were seen in punctate structures homogenously distributed throughout the cytoplasm whereas CCL2-ir formed large fluorescent puncta clustered around the nucleus within neuronal cell bodies (Dansereau et al., 2008). Confocal microscopy revealed the presence of CCL2 immunoreactivity in the dorsal horn of the spinal cord (Fig. 1). CCL2 dorsal horn staining was most intense within the substantia gelatinosa, as well as in a band spanning the boarder between inner lamina II and lamina III. Double-labeling experiments also showed that CCL2 co-localized with substance P and CGRP positive axon terminals in the outer portion of lamina II (Dansereau et al., 2008). In addition CCL2 staining was also concentrated in lamina IIi where both substance P and CGRP-immunoreactivities are sparse. CCL2 is only in primary afferent fibers, not in 2nd order neurons.

The detection of CCL2 in soma and processes of DRG neurons as well as its presence in neuropeptide positive vesicle-like structures in the dorsal horn strongly suggest that CCL2 may be transported and subsequently secreted from neuronal nerve terminals. To test this hypothesis, the release of CCL2 was examined in isolated dorsal horn preparation following KCl or capsaicin stimulation, which are known to induce neurotransmitter release. KCl or capsaicin induced a significant increase of CCL2 in the superfusate over basal outflow. Both KCl- and capsaicin-induced increases in CCL2 release were abolished by cosuperfusion with a calcium chelating agent, demonstrating the calcium-dependent mechanism for the release of CCL2 (Dansereau et al., 2008). These results corroborate recent data obtained by Miller et al. in which it was demonstrated in vitro that CCL2 is located in secretory vesicles and released upon depolarization by CCL2-EGFP transfected DRG-F11 neuronal cell line (Jung et al., 2008).

The ability of CCL2 to stimulate nociceptive neurons suggested its involvement in pain sensitivity modulation (Sun et al., 2006). Intrathecal administration of exogenous CCL2 induces a rapid and sustained hyperalgesia in the hot-plate test provoking profound hindpaw mechanical allodynia (Dansereau et al., 2008). These changes in non-noxious tactile threshold, previously observed on a short period of time (Tanaka et al., 2004), were maintained over 4 days after a single CCL2 i.t. administration (Dansereau et al., 2008). Importantly, these pronociceptive effects of CCL2 were clearly mediated via CCR2, as concomitant injection of CCL2 with the CCR2-selective antagonist INCB-3344 (Brodmerkel et al., 2005) completely blocked CCL2-induced hyperalgesia and allodynia (Dansereau et al., 2008). These behavioral observations are further supported by genetic evidence, since mice overexpressing CCL2 exhibited enhanced nociceptive behavior in responses to both thermal and chemical stimulus modalities (Menetski et al., 2007). Altogether, these data demonstrate that CCL2 enhances sensitivity to pain by direct action on CCR2 expressed by nociceptive and/or spinal neurons and suggest that CCL2/CCR2 signaling plays an important role in the establishment and/or persistence of pain. Finally, a CCR2 antagonist blocks CCL2 nociceptive effects, suggesting that CCR2 represents a target for the development of new therapeutic drugs for pain relief.

In summary, CCL2 is constitutively expressed by uninjured rat DRG and provide evidence for a direct implication of CCR2 in CCL2-induced acute nociception at the spinal level. Based on these data, we would like to propose a neuronal mechanism by which CCL2 might modulate acute pain in healthy animals through its receptor CCR2. This mechanism entails synthesis of CCL2 in DRG neurons where it appears to be stored in both neuropeptide-containing and neuropeptide-free vesicles and may be released in a calcium-dependent and sustained manner from both DRG neuronal cell bodies and their projections in the dorsal horn of the spinal cord. In the DRG, CCL2 may directly excite primary nociceptive neurons by autocrine and/or paracrine processes, perhaps participating to the intraganglionic cross-excitation phenomenon. In addition to this mechanism, CCL2 synthesized in DRG neurons, is released at the spinal dorsal horn to modulate the activity of post-synaptic neurons and glial cells, therefore facilitating pain transmission.

2. Role of CCL2-CCR2 signaling in triggering spinal neuroimmune reaction to peripheral nerve injury

The fact that nerve injury can induce spinal microglial activation has been demonstrated in several models of pain hypersensitivity (Colburn et al., 1999, Fu et al., 1999, Tsuda et al., 2003, Zhuang et al., 2005, Scholz and Woolf, 2007). Activated microglia have been known to release a number of pronociceptive substances, making them a key player in the pathophysiology of neuropathic pain (Watkins and Maier, 2003, Marchand et al., 2005, Tsuda et al., 2005, Scholz and Woolf, 2007). Unlike peripheral nerve injury, rhizotomy produces only a weak microglial reaction within the spinal cord gray matter (Scholz et al., 2008). This

indicates that degeneration of central terminals itself (e.g. formation of cellular debris) does not account for the inflammatory response in the spinal dorsal horn after peripheral nerve injury and that activation of microglia must occur in response to a cell-to-cell signaling mechanism. The initiating signaling mechanism has remained elusive however. Several candidates can be considered. For example, ATP has been a likely candidate. ATP release from several sources is associated with tissue damage, neuronal and glial signaling (Salter et al., 1993, Tsuda et al., 2005). In particular, specific P2X4 receptor expression occurs on microglia following nerve injury (Tsuda et al., 2003) and ATP-stimulation of microglia causes the release of brain-derived neurotrophic factor (BDNF), which, in turn, acts on neuronal (tropomyosin-related kinase-B) TrkB receptors to alter chloride homeostasis (Coull et al., 2005), impairing inhibition and raising neuronal excitability (De Koninck, 2007). Microglial chemotaxis by ATP via P2Y12 receptors has also been proposed as an additional candidate (Honda et al., 2001, Haynes et al., 2006, Kobayashi et al., 2008, Tozaki-Saitoh et al., 2008). However, while both pharmacological blockade or knock-down of P2X4 receptors (Tsuda et al., 2003) or of P2Y12 receptors (Tozaki-Saitoh et al., 2008) prevent the development of nerve injury-induced tactile allodynia, they do not prevent the increase in OX-42 immunoreactivity in the spinal cord, indicating that other signaling mechanisms, upstream of ATP signaling, are necessary for microglial activation. Cathepsin S-Fractalkine-CX3CR1 is another signaling pathway that is necessary for the full development of nerve injury-induced tactile allodynia (see below). Yet, because it is activated microglia that appears to release Cathepsin S, this pathway is likely not the initial signal causing microglial activation. Another, upstream signaling event must occur that triggers microglial activation, which may be amplified and perhaps maintained by Cathepsin S-Fractalkine-CX3CR1 as well as by ATP signaling.

An emerging candidate as a signaling molecule between injured sensory neurons and spinal microglia is CCL2 that specifically attracts monocytes to the sites of inflammation. CCL2 upregulation was found to be induced in dorsal root ganglion (DRG) neurons by chronic constriction of the sciatic nerve (Tanaka et al., 2004, Zhang and De Koninck, 2006). The CCL2 induced in DRG neurons is transported to the spinal dorsal horn (Zhang and De Koninck, 2006) and released in the spinal cord in response to electrical stimulation of sensory fibers (Thacker et al., 2008). CCR2, the receptor for CCL2, is normally expressed on cells of monocyte/macrophage lineage in the periphery (Rebenko-Moll et al., 2006) and can be upregulated in spinal microglia by peripheral nerve injury (Abbadie et al., 2003). The spatial profile of upregulated CCL2 expression in the spinal dorsal horn matches that of activated microglia (Zhang and De Koninck, 2006, Beggs and Salter, 2007, Thacker et al., 2008). Temporally, the transient upregulation of CCL2 closely precedes microglial activation as well (Zhang and De Koninck, 2006). Exogenous spinal administration of CCL2 induces spinal microglial activation and this activation is lost in CCR2 knock-out mice (Zhang et al., 2007). Finally, nerve injury induced-spinal microglial activation was prevented in mice lacking CCR2 (Zhang et al., 2007) or by local spinal administration of CCL2 neutralizing antibodies (Zhang et al., 2007, Thacker et al., 2008). Thus, CCL2 secretion by sensory neurons acting on microglial CCR2 appears to be a viable candidate as the initiating cell-to-cell signaling pathway triggering spinal microglial activation following peripheral nerve injury.

Recent results also revealed that nerve injury-induced spinal microglial response may include not only activation of CNS microglia, but also the generation of new microglia from proliferation (Echeverry et al., 2008) and from recruitment of peripheral monocytes (Zhang et al., 2007). Whether this infiltration occurs only under certain conditions is a subject of debate (Soulet and Rivest, 2008), but it also depends on CCL2/CCR2 signaling, because neutralization of CCL2 at the spinal level prevents monocyte infiltration into the parenchyma of the dorsal horn after nerve injury (Zhang et al., 2007) and CCR2 receptor expression on circulating monocytes is necessary for CNS infiltration (Mildner et al., 2007, Zhang et al., 2007). Thus, both resident microglia activation and monocyte/macrophage infiltration appear to be due to

a signaling mechanism originating from the spinal cord. Other evidence implicate CCL2/CCR2 in the recruitment of monocytes/macrophages and activated lymphocytes into the CNS in a variety of inflammatory, infective and traumatic conditions (Kelder et al., 1998, Huang et al., 2001, Rancan et al., 2001). CCL2 may also facilitate chemotaxis from the periphery to the CNS by altering the expression of tight junction-associated proteins in brain and spinal cord microvascular endothelial cells, thereby increasing blood–brain/spinal cord barrier permeability (Stamatovic et al., 2003, Song and Pachter, 2004).

CCL2-CCR2 signaling is not only a necessary mediator for spinal microglial activation, it is necessary for the development of tactile allodynia following peripheral nerve injury, because mice lacking CCR2 do not develop the hypersensitivity (Abbadie et al., 2003, Zhang et al., 2007). Using selective deletion of CCR2 in CNS microglia vs. peripheral monocytes, we also showed that either population was sufficient for the development of full mechanical allodynia (Zhang et al., 2007). Thus, preventing the development of nerve injury-induced mechanical allodynia may require targeting both CNS resident microglia and blood borne monocytes.

3. CCR2: transgenics vs. pharmacology

This section reviews and compares data obtained in CCR2 deficient mice, in mice overexpressing CCL2 and the effects of CCR2 antagonists in models of acute and chronic nociception (Fig. 2). Mice deficient for CCR2 (CCR 2 −/−) were generated by homologous recombination as described in Abbadie et al., (2003). Mice overexpressing the ligand CCL2 (CCL2 tg) have been designed to express CCL2 in an astrocyte/Schwann cell specific manner and relate to previous studies on glial interactions. Recent studies have shown a strong influence of CNS glia on persistent pain states (for review see Abbadie, 2005;Watkins and Maier, 2003;Marchand et al., 2005). The anti-nociceptive effects of two CCR2 antagonists were evaluated. For studies in mice, (3S,4S)-N-((1R,3S)-3-isopropyl-3-{[7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1 H)-yl]carbonyl}cyclopentyl)-3-methyltetrahydro-2H-pyran-4-aminium chloride (gift from Chris Moyes and Lihue Yang, Merck Research Laboratories) was used. For the rat lysophosphatidylcholine (LPC)-induced focal demyelination of the sciatic nerve, two enantiomers were used, the active CCR2 receptor antagonist isomer (R)-4-Acetyl-1-(4-chloro-2-fluorophenyl)-5-cyclohexyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one (CCR2 RA-[R]) or its inactive enantiomer (S)-4-Acetyl-1-(4-chloro-2-fluorophenyl)-5-cyclohexyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one (CCR2 RA-[S]) (gift from Eli Lilly, Bhangoo et al., 2007).

In models of acute nociception, CCR2 deficient mice showed no differences as compared to control mice whereas mice overexpressing CCL2 (CCL2 tg) showed a significant increase in thermal nociception (hot-plate) (Abbadie et al., 2003, Menetski et al., 2007). Similarly, a CCR2 antagonist had no effect on thermal thresholds. In the formalin test, mice deficient for CCR2 showed a decrease in lifting and licking of the inflamed paw (Fig. 2). CCL2 tg mice were hypersensitive to chemical-induced nociception: CCL2 tg mice displayed a significant increase in formalin-induced nociceptive behavior. Interestingly a CCR2 antagonist dose-dependently decreased formalin-induced pain behavior in mice (Fig. 2). This is in agreement with previous studies showing that hyperalgesia is evoked by intraplantar administration of CCL2 (Oh et al., 2001, Abbadie et al., 2003). Several lines of evidence using intracellular electrophysiological recordings in intact DRG, current clamp in vitro electrophysiology, calcium mobilization, neuronal depolarization-induced release of vesicle bound CCL2 and anatomical localization of CCR2 to sensory neurons indicate that chemokines contribute directly to nociception (Oh et al., 2001, White et al., 2005, Sun et al., 2006, Bhangoo et al., 2007, Jung et al., 2008). Most of the neurons that are sensitive to chemokines correspond to nociceptors, since the same neurons also respond to the application of capsaicin, bradykinin, or ATP, substances for which the effects typically are considered as markers for nociceptive neurons.

In a model of inflammatory pain elicited by unilateral intra-plantar administration of complete Freund's adjuvant (CFA), mice deficient for CCR2 showed attenuated hyperalgesia, but CCL2 tg mice displayed both greater edema and thermal hyperalgesia as compared to control mice (Abbadie et al., 2003, Menetski et al., 2007). In CCL2 tg mice, thermal hyperalgesia was significantly different from baseline up to 3 weeks post-CFA. Parallel to these enhanced behavioral responses circulating levels of CCL2 were significantly greater in CCL2 overexpressing mice and remained elevated 7 days post-CFA. Consequently, pro-inflammatory cytokine mRNA (IL-1β, IL-6 and TNFα) levels were greater in skin, dorsal root ganglia (DRG) and spinal cord and the anti-inflammatory cytokine (IL-10) was lower in skin and DRG in CCL2 over-expressing mice than in control mice (Menetski et al., 2007). CCL2 deficient mice showed some impairment of allodynia in the CFA model of inflammatory pain. However, a CCR2 antagonist had no effect in the rat CFA-induced hyperalgesia model, consistent with CCR2 antagonists being not efficacious in rheumatoid arthritis clinic trials.

In a model of neuropathic pain triggered by partial sciatic nerve ligation, mice deficient for CCR2 did not develop mechanical allodynia (Abbadie et al., 2003, Zhang et al., 2007), but mice overexpressing CCL2 developed allodynia to the same extent and duration as compared to control mice (Menetski et al., 2007). Similarly to data obtained in the CCR2 deficient mice, a CCR2 antagonist administered 5 days following partial sciatic nerve injury in mice significantly reversed mechanical allodynia (Fig. 2).

In the rat model of unilateral lysophosphatidylcholine (LPC)-induced focal demyelination of the sciatic nerve, focal peripheral nerve axon demyelination is accompanied by nociceptive pain behavior (Wallace et al., 2003, Bhangoo et al., 2007). Using this model, the degree to which chronic nociceptive pain behavior was assessed and correlated with the neuronal expression of the CCR2/CCL2 chemokine signaling system. Focal nerve demyelination increased behavioral reflex responsiveness to mechanical stimuli between postoperative day 3 and postoperative day 28 in both the hindpaw ipsilateral and to a lesser degree contralateral to the nerve injury in rats, after which it returned to pre-operative threshold levels. The nociceptive behavior was accompanied by a bilateral increase in the numbers of primary sensory neurons expressing the CCR2 chemokine receptor by postoperative day 14. A significant increase in the number of nociceptive neurons expressing CCL2 was also evident at day 7 following nerve injury. Transgenic mice were also used to confirm these anatomical findings. In mice where the CCR2 receptor is tagged with the enhanced green fluorescent protein (EGFP), a focal nerve demyelination resulted in the upregulation of the CCR2 receptor in neurons. Functional studies demonstrated that acutely dissociated sensory neurons derived from LPC-injured animals responded with increased intra-cellular calcium following exposure to CCL2 on postoperative days 14 and 28, but these responses were largely absent by postoperative day7 and 35. On days 7, 14 and 28, rats received either saline or the active CCR2 receptor antagonist isomer R (CCR2 RA-[R]) or its inactive enantiomer S (CCR2 RA-[S]) by intraperitoneal injection (Bhangoo et al., 2007). Treatment with the R active CCR2 antagonist in nerve-injured rats produced stereospecific bilateral reversal of tactile hyperalgesia at days 14 and 28, but not at day 7 post-surgery. In summary, focal nerve injury results in upregulation of the CCR2 chemokine receptor and its ligand, CCL2, in many nociceptive neurons. Furthermore, an antagonist to the CCR2 receptor attenuated the pain behavior at late but not early time points, suggesting the presence of chemokine signaling by both injured and adjacent, uninjured sensory neurons correlating with a persistent behavioral pain state. Therefore, it is possible that chemokine receptor antagonists may be an important therapeutic intervention for chronic neuropathic pain.

In conclusion, the present results showing that the CCL2/CCR2 axis alters nociceptive processing further supports the role of such chemokines in pain processing. The concordance

of data obtained in transgenic mice and antagonist would suggest that inhibiting this axis may result in novel pain therapies, notably for neuropathic pain.

4. Cathepsin S and fractalkine: a new modulatory pathway for neuropathic pain

It is well established that astrocytic and microglial cells modulate the neuronal mechanisms of chronic pain in spinal cord and possibly in the brain (Watkins and Maier, 2003, Scholz and Woolf, 2007). In models of neuropathic pain following peripheral nerve injury, several changes occur at the level of the first pain synapse between the central terminals of sensory neurons and second order neurons. Neuropathic hyperalgesia and allodynia are likely to be mediated by glutamate, substance P and BDNF released by uninjured C fiber terminals with some contribution of substance P released de novo by injured A fibers (Malcangio et al., 2000, Hughes et al., 2007). These neuronal mechanisms can be modulated by pronociceptive mediators released by non neuronal cells such as microglia and astrocytes which become activated in the spinal cord following PNS injury. For instance, agents which inhibit microglial/astrocytic activation attenuate neuropathic hyperalgesia and allodynia (Raghavendra et al., 2003, Clark et al., 2007a). However, the signals that mediate spread of nociceptive signaling from neurons to glial cells in the dorsal horn remain to be established. Recently, the chemotactic cytokine (chemokine) fractalkine has been proposed to subserve such a role (Milligan et al., 2004). The full-length transmembrane protein is expressed on neuronal membranes and the proteolytic cleavage of fractalkine releases a soluble form (Garton et al., 2001) which binds cells expressing CX3CR1, fractalkine cognate receptor. In the spinal cord CX3CR1 is exclusively expressed by microglial cells (Verge et al., 2004, Lindia et al., 2005), suggesting that soluble fractalkine once released by nociceptive neurons could lead to microglial activation. We agree with recent literature showing that spinal injection of soluble fractalkine is pronociceptive in animals (Milligan et al., 2004, Zhuang et al., 2007) via CX3CR1 receptor activation as spinally administered fractalkine is devoid of activity in CX3CR1 −/− mice (Clark et al., 2007b). In microglial cells the activation of CX3CR1 by soluble fractalkine leads to phosphorylation of p38 MAPKinase thereby promoting the generation of pronociceptive mediators including cytokines.

We have recently identified the lysosomal cysteine protease cathepsin S released by activated microglia as responsible for the proteolytic liberation of soluble fractalkine in the spinal cord under conditions of neuropathic pain (Clark et al., 2007b). Using a gene expression analysis approach we have recently found that the mRNA encoding the lysosomal cysteine protease cathepsin S was up-regulated in rat dorsal root ganglia (DRG) following peripheral nerve injury (Barclay et al., 2007). Cathepsin S protein was expressed in infiltrating macrophages in DRG and at the site of injury (Barclay et al., 2007) and in activated microglia in the ipsilateral dorsal horn of the spinal cord in the area where damaged fibers terminate (Clark et al., 2007b). The expression of cathepsin S in dorsal horn microglial cells correlated with the maintenance of pain hypersensitivity (Fig. 3) as cathepsin S immunostaining showed an increase 3 days after nerve injury but peaked at 7 and 14 days and remained high for up to 28 days after injury.

In agreement with a pronociceptive role of microglial cathepsin S we have then observed that spinal injections of LHVS (morpholinurea-leucine-homophenylalanine-vinyl sulfone-phenyl), an irreversible inhibitor of cathepsin S, reversed established mechanical hyperalgesia and allodynia and reduced microglial activation in the dorsal horn (Clark et al., 2007b).

Microglia are established secretory cells that in response to inflammatory mediators can secrete lysosomal cathepsin S which has robust extra-cellular activity at neutral pH. We found that the intrathecal spinal injection of activated rat recombinant cathepsin S induced significant mechanical hyperalgesia and allodynia in rats. This observation suggests that in the spinal cord

of neuropathic rats, endogenous cathepsin S secreted by activated microglia can exert pronociceptive action extracellularly. The search for a potential substrate for cathepsin S using a bioinformatics approach, culminated in the identification of a cleavage site of extra-cellular fractalkine. We provide evidence that sensory neurons in culture express membrane associated fractalkine from which cathepsin S liberates soluble fractalkine. In the spinal cord, spinal administration of cathepsin S liberates pronociceptive fractalkine and induces phosphorylation of p38 MAPK in microglia (Clark et al., 2007b).

In summary, we have established that cathepsin S is the protease responsible for the formation of soluble fractalkine in the spinal cord under conditions of persistent pain. We propose that following peripheral nerve injury, activated microglia release cathepsin S which enzymatically cleaves neuronal fractalkine. In turn fractalkine activates CX3CR1 receptors on microglia leading to a further release of micro-glial mediators thus establishing a positive feed back which may contribute to a chronic pain state.

5. Conclusion

As reported here, chemokines play a key role in coordinating injury associated nociceptive events as they serve to regulate inflammatory responses and can simultaneously act on elements of the nervous system. Within both the central and peripheral nervous system, both spinal microglia and neurons, respectively, could be responsible for chemokine action. The ability of small molecule antagonists of CCR2 to ameliorate ongoing pain hypersensitivity in animal models clearly indicates the importance of chemokine signaling in this behavior. At present, few drugs are effective in treating neuropathic pain and most drugs are dose-restricted by side effects, therefore targeting the chemokine system may provide a novel form of therapeutic intervention into states of chronic pain.

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Abbreviations

CCL2, CC chemokine ligand 2; CCR2, chemokine receptor 2; CFA, complete Freund adjuvant; DRG, dorsal root ganglia; FKN, fractalkine; GFAP, glial fibrillary acidic protein; MCP-1, monocyte chemoattractant protein-1.

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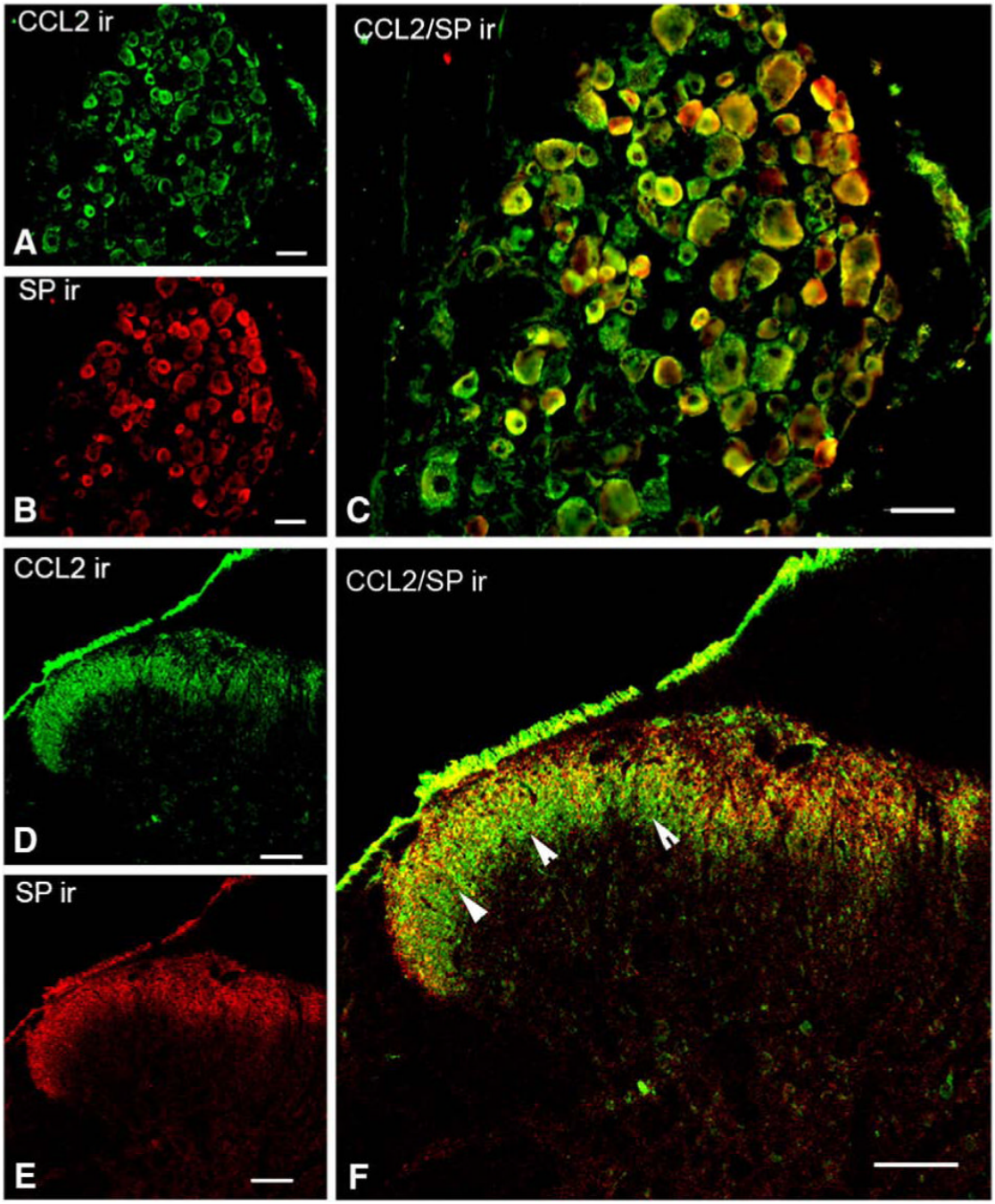
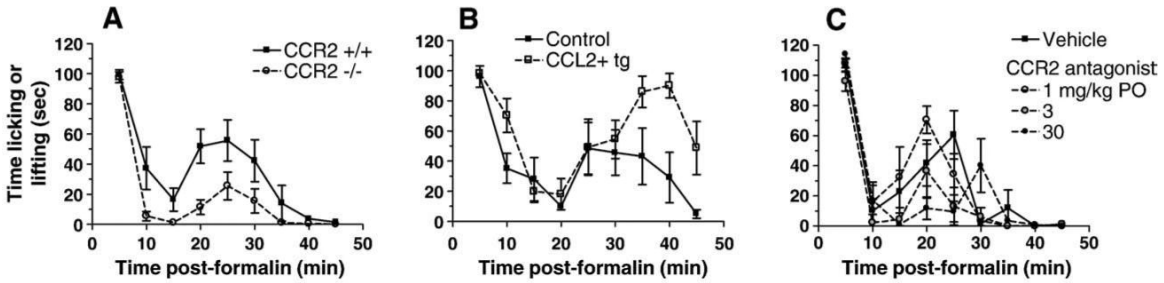


Fig. 1.

Cellular distribution of CCL2 in normal rat dorsal root ganglia (DRG). Double labeling immunofluorescence is used to identify the neurochemistry of CCL2-expressing DRG neurons. Numerous substance P (B, in red) ganglion cells colocalize with CCL2 (A, in green). Merged images show dually labeled cells (C, in yellow). Immunohistochemical localization of CCL2 in normal rat spinal dorsal horn by confocal microscopy. Double labeling of CCL2 (D, in green) with substance P (E, in red); colocalization is shown in yellow (F). A dense network of CCL2-immunopositive processes is observed in laminae II of Rexed. At higher magnification there is no apparent overlap between CCL2 and neuropeptides in the inner portion of laminae IIi (arrowheads). Scale bars equal 100 μm.



D

	CCR2 -/-	CCL2+ tg	CCR2 antagonist
Hot-plate	No change	Increased latency	No change
Formalin	Decreased phase 2	Increased phase 2	Decreased phase 2
Complete Freundís adjuvant	Small decreased in Hyperalgesia	Increased Hyperalgesia	No change
Mouse PSNL Rat LPC	No allodynia	No change	Decreased allodynia
References	Abbadie et al. 2003	Menetski et al. 2007	Neilan et al. 2004 Bhangoo et al. 2007

Fig. 2.

Nociceptive responses in CCR2 deficient mice, in mice overexpressing CCL2 and the anti-nociceptive effects of a CCR2 antagonist. (A–C) Example of CCL2/CCR2 contribution to nociceptive behavior in the formalin test duration of licking and lifting in response to intraplantar formalin injection is significantly reduced in the CCR2 knock-out mice as compared to wild-type mice (A), and is significantly increased in mice overexpressing CCL2 as compared to control mice (B). A CCR2 antagonist significantly decreased phase 2 of the formalin test (C). (D) Summary of data in CCR2 deficient mice, CCL2 overexpressing mice (CCL2+ tg) and effects of CCR2 antagonists in nociceptive pain models.

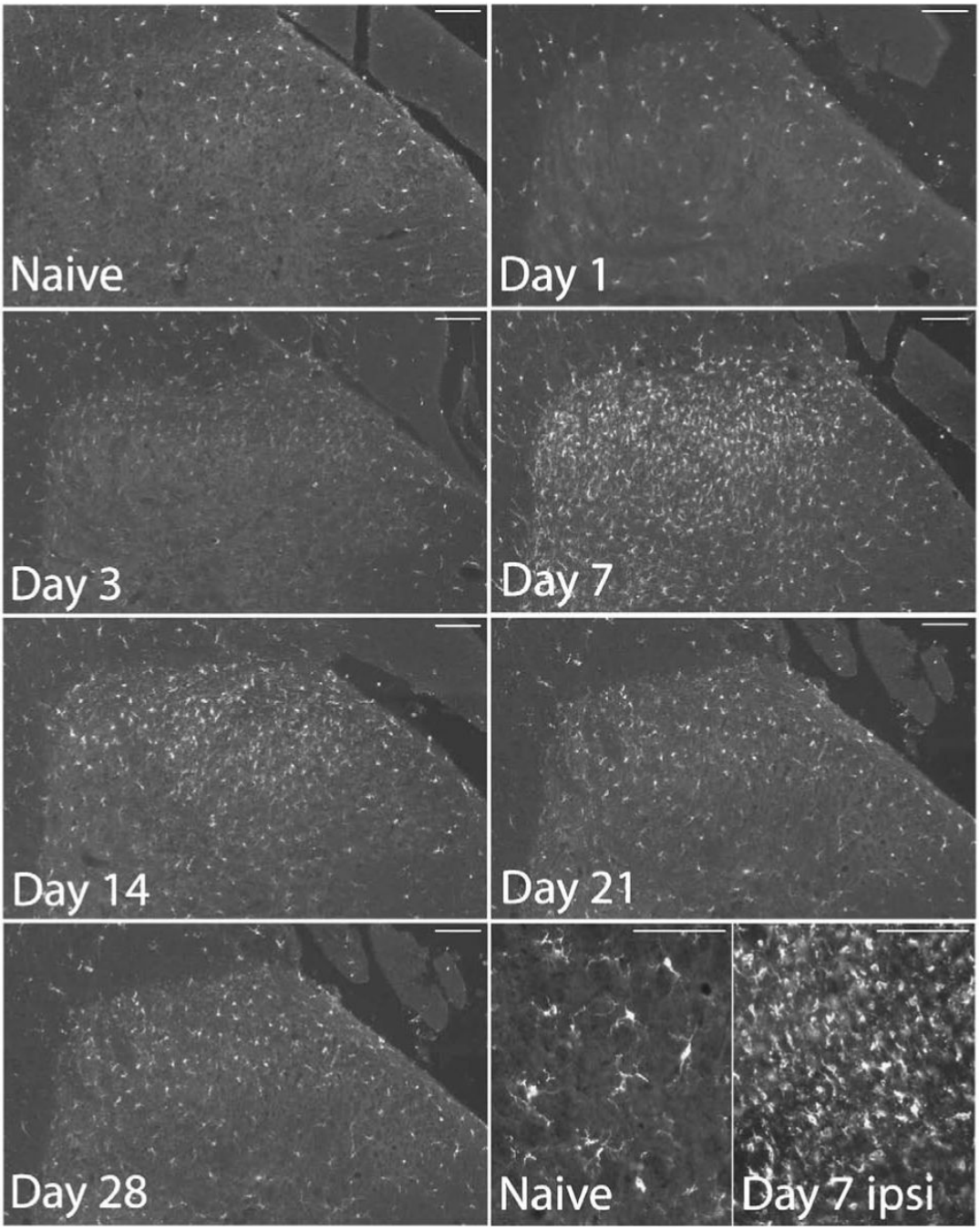


Fig. 3.

Time course of cathepsin S expression in the dorsal horn following peripheral nerve injury. Photomicrographs show increased expression of cathepsin S in the ipsilateral dorsal horn of the spinal cord, peaking at 7 days following partial nerve ligation. Scale bars = 100 μm.