Chemokine mediated neuron–glia communication and aberrant signalling in neuropathic pain states
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Treatment of neuropathic pain is problematic; response to current pharmacological interventions is often poor and associated with undesirable side-effects, thus the identification of new targets for treating this condition is needed. Here we collect evidence demonstrating the potential of chemokines as mediators of neuron–glia communication and contributors to pain signalling. The expression of chemokines such as CX3CL1, CCL2 and CCL21 and their receptors CX3CR1, CCR2 and CXCR3 is altered in the spinal cord under neuropathic pain conditions and chemokine receptor antagonists attenuate neuropathic pain behaviour. By understanding the mechanisms of chemokine-mediated communication we may expose glial targets as a novel approach for the treatment of neuropathic pain.

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Introducing
Neuropathic pain syndromes are chronic dysfunctional pain states arising due to damage in pain signalling pathways, with resulting sensory abnormalities persisting after the initiating pathology has resolved. Although variable in its aetiology, neuropathic pain manifests as spontaneous pain, increased sensitivity to noxious stimuli (hyperalgesia), pain from innocuous stimuli (allodynia), and abnormal unpleasant sensation (dysesthesia) [1].

Despite first being thought of as a disease of purely neuronal origin, pre-clinical studies indicate that the mechanisms underlying the development and maintenance of neuropathic pain involve substantial contributions from the non-neuronal cells of both the peripheral nervous system (PNS) and central nervous system (CNS) [2]. Specifically, as a result of injury to peripheral nerves, microglia and then astrocytes in the spinal cord increase in number, take on an ‘activated’ morphology (large cell bodies and retracted processes) and release pro-inflammatory factors that sensitise neurons [3,4]. The contribution of these cells to central sensitisation following peripheral nerve injury (PNI) has been demonstrated by administration of glial inhibitors that attenuate nociceptive behaviour in animal models of neuropathic pain [5].

Clinically, neuropathic pain is problematic as response to current pharmacological interventions is often poor. Understanding the mechanisms by which neurons and glial cells of the CNS communicate may expose new targets for the treatment of this complex problem. Here we discuss the potential of chemokines as mediators of this communication, as recent evidence has demonstrated that some chemokines play a significant role in the pathogenesis of neuropathic pain.

Chemokines or ‘chemoattractant cytokines’ are a family of small protein molecules, typically 8–10 kDa, obtaining their name from their first recognised function as mediators of leucocyte migration and activation. First discovered in 1987, the chemokines are now a large family of structurally and functionally related molecules capable of interacting with over 20 receptors [5].

The chemokines are named according to the organisation of N-terminal cysteine residues and are divided in to four subfamilies; CC (α-), CXC (β-), C (γ-) and the CX3C chemokines, a family of which there is only one member, CX3CL1 (Figure 1).

Chemokines within each subclass have a promiscuous relationship with their receptors; multiple chemokines can bind to the same receptor and a single chemokine may bind several receptors (although this might not necessarily occur in vivo [6*]), the exception to the rule being the interaction between CX3CL1 and its receptor CX3CR1 that is monogamous. As a result of this relationship the receptor nomenclature is based on the subtype of ligands to which the receptor binds, that is, the CC chemokines bind to CC receptors.

Secreted by glial cells as well as neurons within the CNS, the biological activity of chemokines is exerted via the action of seven transmembrane domain G-protein coupled receptors (GPCRs) found on the surface membrane of target cells [7]. Activation of these receptors...
results in a diverse range of intracellular signalling cascades, including activation of mitogen activated protein kinases (MAPKs), formation of diacylglycerol (DAG) by PKC and release of calcium from intracellular stores via phospholipase C (PLC) (Figure 2). Such diversity in signalling allows chemokines to regulate numerous cellular processes, playing not only an important role in immune cell regulation and the maintenance of homeostasis, but contributing significantly to a number of acute and chronic pathophyslogies, including pain [5].

**CX3CL1/fractalkine**

First described in 1997, CX3CL1 exists in two forms; membrane-bound, tethered to the cell membrane by a mucin-like stalk and as a soluble protein following cleavage [8*].

Under physiological conditions CX3CL1 is constitutively produced by neurons in the brain, spinal cord and dorsal root ganglia (DRG) [9*,10*,11**] through ongoing transcription, membrane insertion and proteolytic release by a disintegrin and metalloproteinase (ADAM10, ADAM17) and cathepsin S (CatS) [12*,13,14]. De novo expression of CX3CL1 has been seen in astrocytes within the dorsal horn of the spinal cord following PNI [15,16]. Conversely, following PNI a decrease in membrane bound CX3CL1 is reported in the neurons of the DRG [17], but not in the dorsal horn of the spinal cord [11*]. The cleavage of CX3CL1 from the neuronal membrane by CatS is vital for the development of pain behaviours following injury [9*]. Accordingly, the level of soluble CX3CL1 in the cerebrospinal fluid of neuropathic animals is increased, but returns to baseline levels subsequent to treatment with CatS inhibitors [14,19].

**Figure 1**

Classification of chemokines according to the organisation of N-terminal cysteine residues.

**Figure 2**

Schematic representation of the main signal transduction pathways activated by chemokines. AC, adenylate cyclase; CaM Kinase, Ca2+/calmodulin-dependent protein kinase; DAG, diacylglycerol; IP3, inositol 1,4,5-trisphosphate; JAK2, janus kinase 2; MAPK, mitogen-activated protein kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C.
The CX3CL1 receptor, CX3CR1, is found predominantly in microglia in the brain and spinal cord where it is constitutively expressed [13,16], and increased significantly following PNI as a result of increased expression and/or proliferation and recruitment of microglia [11*,12*,18]. CX3CR1 is known to be crucially involved in the generation of neuropathic pain, as mice lacking CX3CR1 do not develop allodynia following PNI [18] and administration of a CX3CR1 neutralising antibody attenuates neuropathic allodynia [10*]. However little is known about the mechanism by which the expression of this receptor is upregulated. Recently an interleukin-6 (IL-6) dependent mechanism has been defined; pre-treatment with an IL-6 neutralising antibody prevents the increase in CX3CR1 expression that follows PNI, and intrathecal administration of exogenous recombinant rat interleukin-6 (rIL-6) induces a significant increase in CX3CR1 expression in the spinal cord [20].

In the spinal cord, activation of CX3CR1 results in an increase in intracellular calcium levels [12*], the phosphorylation of the MAPK p38 in microglia [9*,17] and release of pro-nociceptive mediators such as IL-1β, IL-6 and nitric oxide (NO) [10*]. The precise pathway by which p38 becomes phosphorylated is not yet understood, however recent evidence suggests G-protein couple receptor kinase 2 (GRK2) may play a role. GRK2 is an inactivating kinase of p38, preventing its activation by binding to a key threonine residue [21]. The expression of GRK2 is reduced in microglia/monocytes isolated from the spinal cord of neuropathic rats [22,23].

The involvement of the CX3CL1/CX3CR1 pathway in neuron-glia communication is summarised in Figure 3. Neuronal CX3CL1 is liberated by CatS, a lysosomal protease released by microglia following activation of the P2X7 receptor by high concentrations of ATP [24] likely to be neuronal in origin [25]. Soluble CX3CL1 binds to CX3CR1 on microglia resulting in the phosphorylation of p38, ultimately leading to the increased synthesis and release of pro-nociceptive mediators. These diffuse and bind to receptors on dorsal horn neurons, resulting in the hypersensitivity and spontaneous firing that is characteristic of central sensitisation, and also provide a feedback mechanism by which further microglia are activated.

**CCL2/MCP-1**

CCL2, also known as monocyte chemoattractant protein (MCP-1) is amongst of the first human chemokines to be characterised and belongs to a family of four other monocyte attracting chemokines that bear highly homologous structures [26]. Under physiological conditions, CCL2 is thought to be almost absent within the CNS as its mRNA is undetectable in the spinal cord of naive animals [27*,28]. Nonetheless, its constitutive expression has been demonstrated within small and medium neurons of the DRG and in neuronal cells of the superficial lamina of the dorsal horn of the spinal cord [29]. Following PNI, CCL2 upregulation has been demonstrated within non-peptidergic small DRG neurons that co-expressed activating transcription factor 3 (ATF3), a marker of neuronal damage [27*,30*,31,32], as well as within neurons, microglia [28,33*] and, more recently, astrocytes [34] within the spinal cord. As well as the evoked release of CCL2 in the dorsal horn being increased under neuropathic conditions [30*,32], increased release of CCL2 occurs in cultured DRGs following depolarisation with high concentration potassium [35*], suggesting activity dependent release of CCL2 from sensory neurons.

The localisation of CCR2 (CCL2’s primary receptor) within the nervous system is more heavily debated. CCR2 mRNA has been found to be increased at the site of injury and lumbar DRG following PNI, and increased immunoreactivity for the CCR2 protein has been reported in microglia in the dorsal horn of the spinal cord [33*]. This microglial expression is supported by the observation that spinal administration of CCL2 activates microglia (increases OX-42 immunoreactivity and phospho-ERK) [30*]. CCL2 administration also results in phosphorylation of ERK in neurons, which can be prevented by pre-treatment with a CCR2 antagonist [35*]. Neuronal expression of CCR2 is supported by the work of Gao, demonstrating CCL2 is able to induce rapid excitation of dorsal horn neurons in isolated spinal cord slices [34].

However, the presence of CCR2 in microglia has recently been questioned in models of multiple sclerosis. In these studies the development of a CCR2-red fluorescent protein knock-in mouse allowed the identification of two distinct populations of monocyte-derived cells within the CNS following the induction experimental autoimmune encephalitis (EAE) [36,37**]. A CCR2 expressing population of cells was identified that did not express CX3CR1, as CX3CR1 is known to be expressed exclusively in microglia [10*,11**], this suggests microglia might not express CCR2, and CCR2 positive cells are in fact infiltrating monocytes [37**]. Indeed, as there is evidence for macrophage infiltration of the dorsal horn following PNI, these cells might be the source of CCR2 [27*]. CCR2 has also been shown to be upregulated in the astrocytes of animals showing neuropathic behaviours following spinal cord injury [38] and has been visualised in cell bodies of neurons within laminae I–V of the dorsal horn [39].

The CCR2 receptor is coupled to a G-protein of the Gai class [40]. Thus the primary signal transduction pathway of CCR2 is the inhibition of adenylate cyclase and a resultant decrease in intracellular cAMP. There is activation of PLC-β2 and subsequent release of Ca2+.
from intracellular stores via IP3, that triggers signalling cascades including protein kinase C (PKC), calmodulin-dependent protein kinase II (CaMKII), phosphatidylinositol 3-kinase (PI3K), extracellular signal-related kinase (ERK), p38 and Akt, that are involved in cell migration, survival, transcriptional regulation and release of pro-nociceptive molecules. CCL2–CCR2 signalling may also contribute to pain by dis-inhibition of GABA-ergic transmission; co-administration of CCL2 with GABA to cultured neurons results in a
rapid concentration-dependent reduction of GABA-induced inward currents. This effect is not inhibited by omission of GTP from patch-clamp media, suggesting G-protein independence [39].

As summarised in Figure 4, these data suggest a mechanism by which neurons, astrocytes and possibly microglia and/or infiltrating macrophages can interact in vivo. Whilst the precise location of CCR2 is still debated, it is apparent that activation of this receptor by its primary ligand CCL2 plays a key role in the development of neuropathic pain. This is confirmed by pharmacological intervention of CCL2–CCR2 signalling; administration of a CCL2 neutralising antibody or antagonist attenuates pain behaviour [30*] (Figure 4).

Other contenders

There is emerging evidence for a role of several chemokines besides CX3CL1 and CCL2 in pain mechanisms.

CCL21, secondary lymphoid-tissue chemokine (SLC), is expressed in damaged small diameter primary sensory neurons in the DRG [41,42] and is transported to axons within the dorsal horn of the spinal cord [43] where it induces chemotaxis of microglia via activation of the CXCR3 receptor [44]. The administration of a CCL21 neutralising antibody attenuates the development of tactile allodynia in neuropathic animals [42]. Also demonstrating evidence for involvement in pain signalling is CCL5, regulated upon activation normal T-cell expressed and secreted (RANTES); administration of this chemokine into the periaqueductal grey (an area of the brain crucially involved in pain signals) causes hyperalgesia in rats [45]. CXCL12, stromal cell-derived factor-1alpha (SDF-1α), is another possible contender; alongside its primary receptor CXCR4 it is constitutively present in neurons and satellite cells in the DRG and upregulated following induction of a painful neuropathy by antiretroviral therapy. In this model, CXCR4 mRNA and functional protein is increased, shown by increases in intracellular calcium following stimulation of isolated cells with CXCL12. Administration of a CXCR4 antagonist to antiretroviral treated animals has also been shown to attenuate pain behaviours [46]. In addition CXCL12–CXCR4 signalling has been implicated as a player in pain signalling following spinal cord injury, as both proteins are upregulated following lesion to the spinal cord [47] and in opioid-induced hyperalgesia where CXCL12 is upregulated in sensory neurons within the DRG and CXCR4 within glial cells, and hyperalgesia is reduced by a CXCR4 antagonist [48].

Conclusion

It is established that neuronal, microglial and astrocytic cells are able to communicate with one another in vivo to respond to the changing environment that results in pain behaviour following injury to the nervous system. It is likely that many chemokine ligand–receptor pathways contribute to this communication, with effects on cellular activation state and intracellular signalling molecules.
beyond the chemotactic effects typical to these molecules, which contribute significantly to the overall plasticity of the pain system. Thus, centrally penetrant chemokine receptor antagonists constitute a novel therapeutic approach for the treatment of neuropathic pain.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


Enlightening review on the obstacles and success in the discovery of drugs targeting the chemokine receptors.


First paper identifying fractalkine as a new chemokine belonging to a new class of chemokines.


This study identifies cathepsin S as the protease responsible for the liberation of fractalkine chemokine domain in the spinal cord.


Very first evidence for a pro-nociceptive effect of fractalkine directly injected at spinal cord level.


First description of fractalkine and CX3CR1 expression and distribution in nociceptive sensory pathways.


This article provide evidence for fractalkine and CX3CR1 mediation of neuron–immune cell communication.


Evidence for activity-induced release of CCL-2 from sensory neuron central terminals in the dorsal horn.


First paper providing evidence for the role of CCL2 in neuropathic pain through microglial activation in the spinal cord.


Evidence for CCL2 presence in synaptic vesicles.


