Novel Therapeutic Targets in Neuroinflammation and Neuropathic Pain

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There is abounding evidence that neuroinflammation plays a major role in the pathogenesis of neurodegeneration and neuropathic pain. Chemokine-induced recruitment of peripheral immune cells is a central feature in inflammatory neurodegenerative disorders. Immune cells, glial cells and neurons constitute an integral network that coordinates the immune response by releasing inflammatory mediators that in turn modulate inflammation, neurodegeneration and the signal transduction of pain, via interaction with neurotransmitters and their receptors. The chemokine monocyte chemoattractant protein-1/chemokine (C-C motif) ligand (MCP-1/CCL2) and its receptor C-C chemokine receptor (CCR2) play a major role in mediating neuroinflammation and targeting CCL2/CCR2 represents a promising strategy to limit neuroinflammation-induced neuropathy. In addition, the CCL2/CCR2 axis is also involved in mediating the pain response. Key cellular signaling events such as phosphorylation and subsequent activation of mitogen activated protein kinase (MAPK) p38 and its substrate MAPK-activated protein MAPKAP Kinase (MK) MK-2, regulate neuroinflammation, neuronal survival and synaptic activity. Further, MAPKs such as extracellular signal-regulated kinases (ERK), c-jun N-terminal kinase (JNK) and p38 play vital roles in mediating the pain signaling cascade and contribute to the maintenance of peripheral and central neuronal sensitization associated with chronic pain. This review outlines the rationale for developing therapeutic strategies against CCL2/CCR2 and MAPK signaling networks, identifying them as novel therapeutic targets for limiting neuroinflammation and neuropathic pain.

Keywords: neuroinflammation; pain signaling; MCP-1/CCL2; CCR2; MAPK p38, MK-2; MAPK activation

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Monocyte chemoattractant protein-1 (MCP-1/CCL2) and its receptor CCR2 in neuroinflammation and neuropathic pain

Chemokines are implicated in many diseases of the nervous system. Evidence is emerging that chemokines play a role in the physiology of the nervous system, including neuronal migration, cell proliferation and synaptic activity, besides mediating neuroinflammation through the recruitment of leukocytes by their chemotactic activity. Chemokines and their receptors are among the key players responsible for communication between neurons and inflammatory cells, and this cross talk is
crucial for normal neurological functioning. Chemokines may induce neuronal death directly through the activation of neuronal chemokine receptors or indirectly through the activation of microglial killing mechanisms. Evidence of major roles for chemokines and their receptors in diseases of the brain is accumulating and this system is a potential target for treatment of neurodegenerative diseases [1].

The chemokine MCP-1/CCL2 that attracts monocytes and T cells [2] plays a major role in the nervous system. CCL2 has been suggested to trigger firm adhesion of leukocytes to activated endothelial cells by upregulating integrins on monocytes, as well as influence their subsequent diapedesis [3]. Besides modulating the expression of endothelial adhesion molecules, leukocyte integrins and cytokine production during central nervous system (CNS) inflammation, CCL2 also regulates the permeability of the blood-brain barrier by opening or altering tight junctions [4]. It is one of the main effectors driving post ischemic infiltration of monocytes into the brain parenchyma [5]. Expression of CCL2 by reactive astrocytes has been documented in demyelinating multiple sclerosis lesions (MS) [6]. CCL2 expression by glial cells is involved in directing leukocytes to sites of axonal injury in the CNS [7]. Brain micro vessels from Alzheimer’s disease (AD) patients show elevated levels of CCL2 [8]. The absence of CCL2 protein in mice leads to decreased local macrophage recruitment in experimental autoimmune encephalitis (EAE) [9]. CCL2 plays a major role in the recruitment of leukocytes into the subarachnoid space in various types of infectious meningitis [10].

The chemokine CCL2 is an important mediator in many neuroinflammatory and neurodegenerative brain diseases characterized by neuronal degeneration [11, 12, 13]. CCL2 has been found to be upregulated in actively demyelinating MS plaques [14], and its expression is increased in EAE [15]. CCL2 modulates microglial activation and proliferation, thereby contributing to the inflammatory response mounted in the CNS [16]. CCL2 is known to play a role in mediating nerve damage and demyelination of axons by causing an influx of monocytes and T cells in Wallerian degeneration [17]. CCL2 levels are elevated in the cerebrospinal fluid (CSF) of patients with Lyme neuroborreliosis [18], a disease that shows similar clinical signs as that shown by MS [19]. High levels of CCL2 have been found in the CSF and localized in the microglia of the spinal cord of rhesus monkeys infected intracerebrally with *Borrelia burgdorferi*, the spirochete that causes Lyme neuroborreliosis [20], and CCL2 may also possibly contribute to the axonal damage that affects patients with Lyme neuroborreliosis of the peripheral nervous system (PNS) [21].

The induction of neuronal CCL2 during mild impairment of oxidative metabolism caused by microglial recruitment/activation exacerbated neurodegeneration in Thiamine deficiency (TD)-induced neuronal death. Conversely CCL2-knockout (KO) mice were resistant to TD-induced neuronal death, suggesting that the chemokine CCL2 mediates microglial recruitment and neurodegeneration in this model [22]. Interestingly, CCL2-deficient mice showed reduced neuroinflammatory responses and increased peripheral inflammatory responses to peripheral endotoxin insult. Collectively, these data demonstrate a significant role for CCL2 in regulation of brain inflammation after peripheral endotoxemia [23].

CCL2 also participates in pain regulation by directly interacting with sensory neurons and indirectly via peripheral leukocyte activation in the PNS [24, 25]. Moreover, neuropathic pain induced by nerve injury is not elicited in CCR2 gene-deficient mice [24]. It is significant to note that CCL2 has been seen to be elevated in primary sensory neurons after nerve injury, and CCR2 expression has been observed in both dorsal root ganglia (DRG) sensory neurons and Schwann cells, in injured peripheral nerves [26]. The addition of CCL2 to cultured DRG neurons triggered the release of calcitonin gene-related peptide (CGRP), a nociceptor neurotransmitter, from these cells presumably as a result of increased neuronal excitation [26]. The role of CGRP in different models of pain has been well documented [27].

The upregulation of CCL2 in the sensory neurons, satellite glial cells and Schwann cells of DRG from rhesus monkeys when incubated with live *B. burgdorferi* spirochetes that cause Lyme neuroborreliosis (in which patients present with excruciating pain along the spine and limbs due to radiculitis or inflammation in spinal nerve roots) has been recently reported [28]. Besides mediating inflammation in the nerve roots, CCL2 induced in the cells of the DRG may also be involved in the signaling of the pain response in Lyme neuroborreliosis. A recent study indicates that activation of paracrine CCL2/CCR2 signaling between DRG neurons plays a critical role in the development of peripheral neuropathy associated with Taxol treatment for cancer. Blocking CCL2/CCR2 signaling by anti-CCL2 antibody resulted in the prevention of Taxol-induced peripheral neuropathy including mechanical hypersensitivity and loss of intra epidermal nerve fibers, suggesting that targeting CCL2/CCR2 signaling could be a novel therapeutic approach [29]. CCL2 is derived from several types of cells in the peripheral and central nervous systems following nerve injury, and is largely involved in the pathogenesis of neuropathic pain [30]. Further studies focusing on the functions of the chemokine-cytokine network-mediated regulation of neuroinflammation may lead to novel therapeutic strategies against neuropathic pain.

The role of MAPK p38 in neuroinflammation, apoptosis and glutamate mediated excitotoxicity
The MAPKs are a specific class of serine/threonine kinases that respond to extracellular signals such as growth factors, mitogens and cellular stress and mediate proliferation, differentiation and cell survival in mammalian cells. There are distinct groups of MAPKs within mammalian cells including p38 MAPK, ERKs and JNKs [31].

In the CNS, activation of the p38 MAPK pathway constitutes a key step in the development of neuroinflammation. Inflammatory stimuli bind to receptors of the cell surface triggering intracellular signal transduction pathways such as the nuclear factor (NFκB) pathway and the MAPK pathways [32, 33]. Intracellular p38 MAP kinase gets activated and profoundly modulates somatic inflammatory responses. MAPK p38 signaling controls the expression of adhesion molecules, cytokines and chemokines, and a variety of other factors that mediate and control the inflammatory process. Many inflammatory response proteins such as tumor necrosis factor (TNF-α), interleukin (IL-1, interleukin (IL-6), interleukin (IL-8) and CCL2 depend on p38 signaling for their production [34, 35]. The chemokine CCL2 activates the p38 MAPK pathway in cultured rat hippocampal cells [36]. IL-1 induction of neuron apoptosis depends on p38 MAPK activity after spinal cord injury [37].

Apoptosis or cell death following cytokine-mediated glutamate-induced excitotoxicity in the brain is also mediated by p38 MAPK [38]. Pro-inflammatory cytokine expression and p38 MAPK signaling have been implicated in glutamate-induced neuronal death of neonatal rats, and their inhibition may have an important neuroprotective role as part of an anti-inflammatory therapeutic strategy [38]. Excitotoxic neuronal death occurs through the activation of N-methyl-D-aspartate (NMDA) and non-NMDA glutamatergic receptors in the CNS [39]. Glutamate also induces strong activation of p38 and indeed, cell death can be prevented by inhibitors of the p38 MAPK pathway. Furthermore, intracellular signals generated by-aminooxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors activate the stress sensitive MAP kinases implicated in apoptotic neuronal death such as JNK and p38. NMDA and AMPA receptor expression and cortical neuronal death are associated with p38 MAPK in glutamate-induced excitotoxicity in vivo [39]. Neuronal cell death due to glutamate excitotoxicity is mediated by p38 MAPK activation in the rat cerebral cortex [40]. Inhibition of delayed induction of p38 MAPK attenuates kainic acid-induced neuronal loss in the hippocampus [41, 42, 43]. In addition, MAPK p38 participates in the neuronal MAP kinase cascade of signaling mechanisms that integrate synaptic plasticity and memory [44].

Irregularities in p38 MAPK signaling in neuronal cells have been associated with diseases linked with neuroinflammatory processes where persistent inflammatory stimuli such as chronic microglial activation have a damaging rather than a protective effect [45]. Prolonged and sustained activation of glial cells can result in an exaggerated inflammatory response that causes neuronal cell death through the elevated release of proinflammatory cytokines, which have a potential neurotoxic effect leading to neurodegeneration [46, 47]. A role for p38 MAPK activation in mediating astrogliosis has also been documented in Lyme neuroborreliosis [48, 49].

The role of p38 MAPK and its substrate MAPK activated protein MAPKAP Kinase (MK) MK-2 in neuroinflammation

Several p38 MAPK targets are kinases and transcription factors, known to play a role in inflammation via the production and activation of inflammatory mediators. It is known that p38 MAPK becomes activated by many cellular stresses besides inflammatory cytokines [50, 51]. The identification of p38 MAPK (an isoform of p38 MAPK) activation residues [52] and the discovery of MK2 as being a direct substrate of p38 MAPK, provided the first indications of the possible molecular mechanisms that could be involved in the activation of p38 MAPK cascades [53, 54]. MAPKAP Kinases (MKs), MAPKAPK-2 (MK-2) and MAPKAPK-3 (MK-3) are serine/threonine kinases are part of the subfamily that bind to and are specifically activated by the p38 MAPK isoforms [55, 56]. MK-2 is recognized as the most important kinase to be activated by p38 MAPK since it has a vital role in mediating both cellular stress and inflammatory responses. The p38 MAPK/MK-2 complex has been documented to contribute to inflammation in vivo, since MK-2 knockout mice are resistant to endotoxic shock as a result of lipopolysaccharide (LPS)-stimulation [57]. MK-2 is involved in regulating the production of TNF-α, IL-6, IL-8, and several other cytokines that play a role in inflammation [58, 59]. MK-2 expression and activation is increased in microglia that have been stimulated with LPS- and interferon-α, and microglial cells cultured from MK-2 knockout mice showed a reduction in the levels of inflammatory cytokines [60]. This signaling is of particular interest as the p38 MAPK/MK-2 pathway and the consequent production of inflammatory cytokines have a significant role in neurodegenerative disease processes where oxidative stress and persistent neuroinflammation are the primary causes of disease. MAPKAP kinase-2, one of p38 MAPK’s more prevalent substrates has also been implicated in Parkinson’s disease (PD), where it has been shown that MK2-deficient mice show decreased levels of neuroinflammation and loss of dopaminergic neurons within the substantia nigra after treatment with the Parkinson’s inducing neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) compared to MK-2 wild-type mice [61].
MAPK p38 and its substrates have been shown to modulate neuronal plasticity and have been implicated in several neurodegenerative diseases. The release of pro-inflammatory cytokines as a result of activation of the p38 MAPK cascade, has been reported to be involved in AD, PD, MS, cerebral ischemia, depression and neuropathic pain [62]. The activation of p38 MAPK signaling mediates changes in morphology and density of dendritic spines seen during the development of neurodegenerative disease and is associated with memory impairment after epileptic seizures [62]. The understanding of the functional role of the p38 MAPK signaling cascade in affecting synaptic plasticity in the hippocampus and its potential role in neurodegenerative diseases such as AD has made significant progress. Several formulations of p38 MAPK inhibitors are being actively screened in phase II clinical trials for depression. Evaluation of MAPK p38 inhibitors as therapeutic agents in neural diseases is an active area of research and they are being rigorously explored in both preclinical experimental models in addition to clinical trials for various inflammatory diseases [63].

The role of Mitogen activated Protein Kinase (MAPK) signaling in the transduction of the pain response

Pain is a feeling of distress caused by activation of specific nerve endings in response to various stimuli capable of causing tissue injury. This specialized sensory processing, also defined as “nociception” leads to a reflex or withdrawal response that helps to reduce or prevent tissue injury [64]. However, over time, nociceptive sensitization may take on a detrimental role where increased sensitivity can lead to prolonged activation of nociceptors, and the subsequent recruitment of peripheral and central mechanisms may result in persistent pain perception [65]. There is growing evidence that MAPKs such as p38, ERK1/2 and JNK play a critical role in the signal transduction cascade associated with chronic nociception [66, 67]. The activation of MAPKs mediated by inflammatory stress stimuli, neurotransmitters, growth factors and hormones, mediate diverse intracellular responses operative both at the transcriptional and post-transcriptional levels [68]. Protein kinase p38 MAPK is also a potential target for the treatment of pathological pain [69]. Activation of p38 MAPK in spinal microglia is a critical link in inflammation-induced spinal pain processing [70], and its activation in uninjured primary afferent neurons and in spinal microglia contribute to the development of neuropathic pain induced by selective motor fiber injury [71].

Experimental pain models have shown sustained MAPK activation in nociceptive primary sensory neurons present in the DRG, second order neurons located in the dorsal horn of the spinal cord, neurons located in the brainstem and thalamic-cortical regions that are important for nociceptive processing and interpretation of pain related signaling information [72, 73]. Importantly, inhibition of MAPK phosphorylation, both of ERKs and p38, resulted in the production of antinociceptive activity in experimental pain models [69]. MAPK activation is considered to be an essential component of nociceptive peripheral and central sensitization and proposed to function as a “master switch” in the initiation and maintenance of pathological pain. Increased MAPK phosphorylation (activation) has been observed in neurons and glia in peripheral nerve fibers, DRG cells, and the spinal cord dorsal horn and supraspinal areas relevant to pain-processing [74]. Studies have indicated that topical application of capsaicin to the hind paw results in the increased phospho-p38 (activated) immune- reactivity in the nerve fibers of the dermis [75]. Further, intraplantar injection of capsaicin induced phosphorylated ERK in intra-epidermal nerve fibers and nerve bundles, which was subsequently prevented when pretreated with MAPK kinase enzyme (MEK) inhibitor [76]. Surgical models of neuropathic pain revealed differential activation of p38, ERK and JNK in neurons and glia of DRG that were in turn attenuated by using inhibitors that block MAPK phosphorylation [72, 77, 78, 79, 80, 81, 82].

MAPK p38 has been the subject of extensive efforts in basic research and drug discovery [83]. Inhibition of p38 MAPK and ERK differentially modulates cytokine expression [41]. MAPK p38 is a key signaling molecule and a therapeutic target for inflammatory diseases such as arthritis [32]. A specific inhibitor of p38 MAPK, SB203580 (Calbiochem) was effective in affording brain protection with a wide therapeutic window against focal ischemic insult in rats by suppressing the expression of pro-inflammatory cytokines [84]. Considering the central role that MAPK signaling, particularly p38 MAPK plays in orchestrating the immune response, glutamate-mediated excitotoxic neuronal and glial damage, modulation of synaptic function and the signal transduction of the pain response, MAPK signaling cascades serve as novel targets for the development of therapeutic strategies aimed at treating neuroinflammatory diseases that result in neurodegeneration, demyelination as well as neuropathic pain.

Concluding remarks

Accumulating evidence suggests that there are major roles for the chemokine MCP-1/CCL2 and its receptor CCR2 in inflammatory neurodegenerative disease, in addition to the signal transduction of the pain response. The MAPK p38/MK-2 signaling cascade has fundamental roles in mediating neuroinflammation, modulating neuronal and glial survival and apoptosis, particularly via glutamate mediated excitotoxicity, besides regulating neuronal plasticity. Although the pathological processes responsible are not fully understood, there is substantial evidence that
activation of MAPKs is critical to peripheral and central sensitization associated with conditions of nociceptive inflammatory and neuropathic pain. Crosstalk between neurons and glia is an essential component of pathogenic pain development and increased MAPK phosphorylation in neurons and glia of the DRG, spinal cord and cortex is considered to be a biochemical marker of pain signaling. Nociceptive-induced cellular signaling in experimental pain models, specifically MAPK phosphorylation, can be utilized to provide mechanistic insights into drug-target interaction along the nociceptive pathways. Together, CCL2/CCR2 and MAPK signaling networks represent novel therapeutic targets for the development of therapeutic strategies aimed at limiting neuroinflammation and neuropathic pain associated with neurological disease states.

Conflicting interests
The authors have declared that no competing interests exist.

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