Glial Mechanisms of Neuropathic Pain and Emerging Interventions

Daehyun Jo, M.D., Ph.D., C. Richard Chapman, Ph.D., and Alan R. Light, Ph.D.

Pain Research Center, Department of Anesthesiology, School of Medicine, University of Utah, Salt Lake City, UT 84109 USA

Neuropathic pain is often refractory to intervention because of the complex etiology and an incomplete understanding of the mechanisms behind this type of pain. Glial cells, specifically microglia and astrocytes, are powerful modulators of pain and new targets of drug development for neuropathic pain. Glial activation could be the driving force behind chronic pain, maintaining the noxious signal transmission even after the original injury has healed. Glia express chemokine, purinergic, toll-like, glutaminergic and other receptors that enable them to respond to neural signals, and they can modulate neuronal synaptic function and neuronal excitability. Nerve injury upregulates multiple receptors in spinal microglia and astrocytes. Microglia influence neuronal communication by producing inflammatory products at the synapse, as do astrocytes because they completely encapsulate synapses and are in close contact with neuronal somas through gap junctions. Glia are the main source of inflammatory mediators in the central nervous system. New therapeutic strategies for neuropathic pain are emerging such as targeting the glial cells, novel pharmacologic approaches and gene therapy. Drugs targeting microglia and astrocytes, cytokine production, and neural structures including dorsal root ganglion are now under study, as is gene therapy. Isoform-specific inhibition will minimize the side effects produced by blocking all glia with a general inhibitor. Enhancing the anti-inflammatory cytokines could prove more beneficial than administering proinflammatory cytokine antagonists that block glial activation systemically. Research on therapeutic gene transfer to the central nervous system is underway, although obstacles prevent immediate clinical application.

Key Words: astrocytes, mechanisms, microglia, neuropathic pain, therapeutic strategies.

Neuropathic pain is a debilitating chronic pain condition accompanied by significant pathological changes in the nervous system. It can arise from injury of the central nervous system (CNS), but it occurs more frequently in association with injury to the peripheral nervous system. Postherpetic neuralgia, a familiar example, results from changes in peripheral and central nervous system somatosensory processing, and it can persist for months or more after the skin lesion has healed. The cardinal signs of neuropathic pain are: 1) hyperalgesia, an increased sensitivity to normally painful stimuli; and 2) allodynia, a painful response to a usually non-noxious stimulus, either static or mechanical. These distressing conditions prohibit the patient with neuropathic pain from leading a normal life.

Pain normally begins at the periphery with injury activation of C- or Aδ nociceptive fibers that carry the excitatory impulses. These impulses induce excitatory postsynaptic potentials in central-transmission neurons located in the dorsal horn of the spinal cord, with subsequent synaptic relays extending to multiple structures in the limbic brain and cerebral cortex. Pain is a conscious experience arising from a redundant, multi-pathway, dynamic system within which profound signal suppression or enhancement can occur at any level of synaptic communication.

Glial cells have recently gained attention as powerful modulators of pain and are emerging as new targets for...
drug development. Activated glia release multiple signaling molecules that enhance central processing of noxious signaling. Glial signaling has pro-inflammatory effects on both nervous and immune system structures, sometimes with pathological effects including neuronal hyperexcitability, neurotoxicity, neuronal plasticity and chronic inflammation. However, glial activation in the CNS may also have beneficial effects that contribute to a local immune environment, such as the release and maintenance of anti-inflammatory factors, restoration of normal noxious signal transmission and protection against neurotoxicity.

Even with advanced treatments, neuropathic pain is often refractory to intervention because of the often complex etiology and an incomplete understanding of the mechanisms behind this type of pain. Despite progress in analgesic drug discovery, the need for therapeutic agents capable of blocking enhanced noxious signal transmission without impairing normal abilities remains largely unmet. Therefore, new therapeutic strategies are needed such as targeting the glial cells, novel pharmacologic approaches and gene therapy. According to recent findings that highlight the active participation of glial cells in the onset and/or maintenance of chronic pain, glia could be potential targets for neuropathic pain control. Moreover, for patients who fail interventional or pharmacological therapies, gene therapy may promise improved pain control, although currently it is primarily experimental.

In this review, our focus is on the dorsal horn of the spinal cord. We describe briefly several potential mechanisms of neuropathic pain involving glia at the level of the CNS. We also describe current pharmacological trials targeting the glia, and discuss the possibilities of gene therapy.

### PART I: BASIC SCIENCE

Nociceptive transmission is normal physiology mediated by multisynaptic pathways, and it initiates processing at multiple sites in the CNS. When injury or inflammation is prolonged, ongoing excitation of primary nociceptive neurons can cause chronic pain. Altered activity in spinal cord and/or brain neurons is relevant in noxious transmission, as well as abnormal firing patterns in primary sensory nociceptors, leads to chronic neuropathic pain. Sustained tissue injury or inflammation in the CNS can also sensitize neurons in the spinal cord, leading to chronic pain. Nerve damage evokes a cascade of immune responses, which may play a role in initiating neuropathic pain, maintaining neuropathic pain acutely and/or play a role in chronic neuropathic pain. No nociceptors have a rich variety of immune receptors, and evidence exists for roles of the interleukins (IL-1β, IL-6 and IL-8), TNF-α, bradykinin, prostanooids, and other. Although epinephrine produces mechanical hyperalgesia and sensitizes DRG mediated by both the protein kinase A and C pathways, and reduced potassium chloride co-transporter expression and increased GABA release contribute to hyperalgesia, pro-inflammatory substances, in particular, pro-inflammatory cytokines (TNF-α, IL-1, IL-2, and IL-6), cyclooxygenase-2, and inducible nitric oxide synthase (iNOS) promote the activation of microglia and the production of inflammatory mediators that acting on nociceptive pathway, lead to central sensitization as well as peripheral and hence, to the development and maintenance of persistent pain states.

1. Central immune system

Peripheral nerve injuries such as complex regional pain syndrome that lead to neuropathic pain states cause not only pathology in the damaged peripheral nerve and dorsal root ganglion (DRG), but also changes in the central processing of sensory information that are best characterized at the spinal level. Nerve injury induces the release of calcitonin gene related peptide (CGRP), substance P, glutamate and adenosine triphosphate (ATP) from the presynaptic terminals of the primary afferents and these also activate glial cells, provoking them to produce pro-inflammatory cytokines, ATP and other neuroactive factors that may further increase neuronal excitability. Such glial activation could be the driving force, maintaining the noxious signal transmission even after the original injury has healed.

2. Glial cells

Glia are non-neuronal cells that provide support and nutrition for neurons, maintain homeostasis, form myelin, and participate in signal transmission in the nervous
system. Microglia and astrocytes are key mediators of the CNS innate immune response to injury, which resembles many aspects of the classical immune defense response. They express several receptors that enable them to respond to neural signals, and they can modulate neuronal synaptic function and neuronal excitability. Glial cells also produce numerous neuroactive mediators such as proinflammatory cytokines and growth factors. Recent findings suggest that activated glial cells can maintain chronic pain, and might therefore be potential targets for treatment. 12) 

3. Microglia

Microglia act as the main form of active immune defense in the CNS, and they are the main source of inflammatory mediators (IFMs: e.g., IL-1β, IL-6, TNF-α, PGE2, NO, BDNF) in the nervous system. These cells are most likely specialized monocytes and as such they can act as phagocytes and replicate themselves when activated. Normally, microglia are constantly monitoring and analyzing the CNS for damaged neurons, plaques, infectious agents and rogue glia. 13) Due to the unavailability of antibodies from the rest of body (antibodies are too large to cross the blood brain barrier), microglia must be able to recognize foreign bodies, ingest them, and act as antigen-presenting cells that activate T-cells. Nerve injury induces extensive proliferation of spinal microglia and related gene expression, such as the microglial markers (e.g. CD11b, TLR4 and CD14) within several hours as well as a dramatic, rapid upregulation of bFGF mRNA. Specifically, nerve injury upregulates several receptors, such as the chemokine receptor CX3CR1 and ATP receptor P2X4 and P2Y12 in spinal microglia. 14-18) The chemokine receptor CCR2 and Toll-like receptor-4 are also expressed in spinal microglia and contribute to neuropathic pain sensitization. 19,20) 

Fractalkine is a unique chemokine that plays an important role in mediating neural-glial interaction. Also, fractalkine acts on CX3CR1 receptor to release IL-1β from spinal microglia. Nerve injury induces a marked cleavage of fractalkine in the DRG, which bind to its upregulated CX3CR1 receptor in spinal microglia, and then it leads to the activation of the p38 MAPK signaling cascade. 18) p38 MAPK, which is activated by upstream kinase MKK3/MMK6, is regarded as a stress-induced kinase and plays a critical role in inflammatory responses. p38 regulates the synthesis of numerous inflammatory mediators via transcriptional regulation.21) For example, p38 activates phospholipase A2 (PLA2) via its downstream kinase MAPKAP-2 (MAPK activated protein kinase-2). PLA2 plays an important role in inflammatory responses. The activation of PLA2 results in the genesis of arachidonic acid for prostaglandin production, which can be catalyzed via cyclooxygenase (COX) to produce prostaglandin E2 (PGE2). The acid-induced increase in PGE2 production is mediated by activation of COX-2 but not by COX1, and acid-induced COX-2 expression and PGE2 production depend on an increase in cytosolic Ca2+ and sequential activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (e.g. NOX5-S) and NF-κB.22) The study of COX expression in the spinal cord using immunohistochemistry showed that COX-1 was expressed in microglia at 1 day to 2 weeks and COX-2 was expressed in neurons at only 2 hours after peripheral formalin injection, which means that spinal microglia activation may play a role in long-term hyperalgesia through the increased expression of COX-1.23) Like fractalkine, p38 activation also causes a rapid release of IL-1β from spinal microglia. p38 activates the transcription factor NF-κB in cultured microglia,24) leading to the expression of the IL-1β, IL-6, COX-2.25) Thus, the fractalkine-CX3CR1-p38 signaling cascade appears to be a mechanism of neuropathic pain (Fig. 1). 

ATP acts as a widespread gliotransmitter in signal translation between neuronal and glial circuits and within glial networks. When released from glia, ATP triggers and maintains glial calcium signals and calcium waves as well as signals to the neurons. The P2X and P2Y ATP receptors constitute a family of calcium-permeable non-selective cation channels gated by extracellular ATP. The P2Y6, P2Y12 and P2Y13 subtypes are highly expressed in microglia and they induce inflammation and microglial activation. 17) Tsuda et al. 18) showed that the activation of microglia in neuropathy requires P2X4 receptors. However, Raouf et al. 26) raised the question of: 1) whether the upregulation of P2X4 receptors takes place downstream of signaling events specifically triggered in conditions of
Fig. 1. Activation and response of microglia in neuropathic pain. Nerve injury upregulates multiple receptors, including the chemokine receptors CX3CR1, P2X/Y, CCR2 and TRL4 in spinal microglia, and it induces the release of fractalkine and neurotransmitters from the primary afferent neuron that also activate microglia. Activation causes microglial cell increase intracellular calcium, and initiates the p38 MAPK/ERK pathway, which is necessary for the release of pre-inflammatory substances. The activated microglia release several pro-inflammatory cytokines, chemokines and other agents that modulate neural transmission by affecting presynaptic release of neurotransmitters and/or postsynaptic excitability. AMPA: \(\alpha\)-mino-3-hydroxy-5-methyl-4-isoxazole propionic acid, ATP: adenosine triphosphate, CCL2: cystein chemokine ligand 2, CCR2: chemokine ligand 2 receptor, ERK: extracellular signal regulated kinase, FRK: fractalkine, HSP: heat shock protein, IFMs: inflammatory mediators, IL-1 \(\beta\): interleukin-1beta, IL-6: interleukin-6, IL-18: interleukin-18, LPS: lipopolysaccharide, MAPK: mitogen activated protein kinase, NF- \(\kappa\)B: nuclear factor- \(\kappa\)B, NKR: neurokinin receptor, NMDA: N-terminal-D-aspartate, NO: nitric oxide, PGE\(_2\): prostaglandin E\(_2\), PLA2: phospholipase A2, TLR: toll like receptor, TNF \(\alpha\): tumor necrosis factor \(\alpha\).
another MAPK family member, is also activated in spinal microglia at early stages of neuropathic pain development. The activation of ERK is necessary for neuropathic pain sensitization. In acute and inflammatory pain conditions, ERK is activated in dorsal horn neurons, and this contributes to the induction and maintenance of dorsal horn neuron sensitization and pain hypersensitivity.

Peripheral nerve injury triggers glutamate release in synapses that leads to the activation of α-amino-3 hydroxy-5 methyl 4 isoxazole propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptor. Such postsynaptic sensitization activates ERK at distal synaptic sites, and activated ERK at synaptic sites may contribute to AMPA receptor modulation, ion channel modulation and other synaptic modifications.31) Phosphorylated ERK (pERK), which is the active form, is induced in spinal astrocytes at late times after spinal nerve ligation. Zhuang et al. reported that a widespread induction of pERK in spinal microglia that peaks between 1 and 3 days post-surgery, expressed both in astrocytes and microglia on day 10, and in dorsal root ganglions on day 10 and 21. It is likely that the ERK and p38 pathways can act together to regulate neuropathic pain. Therefore, some speculate that microglia might be responsible for the first step in a cascade of immune responses of neuropathic pain states, and astrocytes may be involved in their maintenance. However, according to the Colburn et al.’s report, astrocytes could also be involved in initiation of neuropathic pain.32)

Monocyte chemoattractant protein-1 (MCP-1, also named CCL2) and CCR2 (the receptor for MCP-1) play a critical role in such a cell influx in the affected spinal cord. MCP-1 is not present in normal CNS, however, it is dramatically upregulated in DRG and spinal cord after peripheral nerve injury.33,34) MCP-1 production by damaged neurons after peripheral nerve injury may then trigger chemotaxis through its cognate receptor CCR2, expressed selectively on cells of monocyte/macrophage lineage in periphery and in resident and bone marrow-derived microglia.33,34) CCL2 is secreted from neurons upon capsaicin- or K+ dependent depolarization in a Ca2+ dependent and an activity-dependent manner.36,37) Spinal cord expression of CCR2 results in the infiltration of macrophages into the spinal meninges that differentiate into microglia on further parenchyma penetration. Expression of CCL2 in the superficial lamina of the spinal cord dorsal horns triggers spinal astrocyte and microglia activation.34,35) Whereas the activation of microglia surrounding CCL2-expressing spinal cord neurons peaked by day 14 after peripheral nerve injury, astrocytes surrounding CCL2-expressing spinal cord neurons remained robustly activated throughout the entire testing period of 150 days after nerve injury (Fig. 1).34)

Glia express a wide range of pattern-recognition receptors, including toll-like receptors (TLRs). In the CNS, TLR4 is expressed on microglia and perhaps astrocytes and endothelial cells, but not on neurons.39) Also, damaged sensory neurons activate glial cells via TLR2.40) The endotoxin lipopolysaccharide (LPS) is a well known exogenous ligand for TLR4, whereas among its potential endogenous ligands there are heat shock proteins (HSP).41) Cell surface TLRs seems to work in concert to induce innate immune response to microbial membrane components (Fig. 1).

Another unique role for cell surface TLRs is to sense tissue damage by responding to endogenous ligands released from damaged tissues or necrotic cells. Upon activation, TLRs initiate immune-like processes, such as the release of pro-inflammatory cytokines and phagocytosis. TLR4s are predominantly expressed by microglia but can under some circumstances be expressed by astrocytes. The activation of TLR4 induces the release of IL-1β, TNF-α and IL-6. TLR activation in the CNS modulates glial-neuronal communication, creating an excitatory positive-feedback loop in the pain pathway. Hutchinson et al.42) reported that neuron to glia signaling through TLR4 is important not only for initiating neuropathic pain but also for maintaining established neuropathic pain.

4. Astrocytes

Astrocytes are star-shaped glial cells in the brain and spinal cord that assist neurons through the secretion or absorption of neural transmitters. Astrocytes, developmentally derived from the neuroderm, are the most abundant glial cell type in the CNS. In addition to their neuron-supportive functions, astrocytes also directly alter neuronal
communication because they completely encapsulate synapses and are in close contact with neuronal somas. Astrocytes express ionotropic non-NMDA and NMDA receptors as well as metabotropic glutamate, purinergic and substance P receptors. Astrocytes are usually identified by their markers such as glial fibrillary acidic protein (GFAP), vimentin and S-100 $\beta$. Increased density of GFAP staining results primarily from hypertrophy of astrocytes, and it is correlated with the degree of hyperalgesia. The NMDA receptor is partly responsible for GFAP expression. Astrocytes have a characteristic networks, gap junctions, which exist at opposing plasma membranes of many cell types, and contribute to local metabolic homeostasis and synchronization of cellular activities by allowing direct intercellular movements of ions, metabolites and second messenger molecules up to 1,000 Dalton. It is noteworthy that the gap-junction blocker carbenoxolone suppresses the spread of pain.

Astrocytes are essential for energy metabolism in neurons, and they provide a physical link to the vasculature through their processes. Increases in astrocytic Ca$^{2+}$ are associated with vasodilation, which depends primarily on COX-1 metabolites. Study of the effect of maximally stimulated sodium pump activity on the rate of energy metabolism in cerebral astrocytes showed that astrocytes are able to restore the Na$^+$ gradient very rapidly following even large perturbations and contribute significantly to regional variations in glucose consumption associated with functional activity in the brain. A sodium pump activation mediates the effect of Na$^+$ entry on astrocytes energy metabolism.

Astrocytes express the glutamate transporter 1 (GLT-1) and glutamate-aspartate transporter (GLAST) which provide glutamate removal from synaptic clefts and the extracellular space. Nerve injury produces a persistent downregulation of the transporters, after an initial rise. Downregulation might decrease glutamate uptake and subsequently increase excitatory synaptic transmission (Fig. 2).

Spinal cord injury induces an immediate increase in plasma endothelin (ET) levels and a sustained increase in tissue ET levels. ET-1 also induces hypertrophy of astrocytes. ET receptor-B (ET$_B$) is induced in spinal astrocytes after spinal cord injury (Fig. 2).

In acute and inflammatory pain conditions, ERK is activated in dorsal horn neurons, which contributes to the induction and maintenance of dorsal horn neuron sensitization and pain hypersensitivity. Phosphorylated ERK (pERK), which is the active form, is found in microglia on day 2, in both microglia and astrocytes on day 10, and in astrocytes only on 21. These increase the synthesis of inflammatory factors such as IL-1$\beta$, IL-6, TNF$\alpha$, prostaglandin E2 and nitric oxide, which ultimately alter glial glutamate transporter function and gap-junction proteins that facilitate astrocyte-astrocyte activation. Spinal inhibition of this late-phase activation of ERK by intrathecal MAPK/ERK inhibitor (MEK kinase inhibitor) reverses mechanical allodynia, supporting a role of astrocytes ERK in the maintenance of neuropathic pain.

The C-Jun N-terminal kinase (JNK) is the signaling molecule activated consistently in spinal astrocytes in chronic pain conditions. JNK activates c-Jun by phosphorylation to form p-c-Jun, which localizes to GFAP-expressing astrocytes. Spinal nerve ligation in animal models induces a marked increase in pJNK-immunoreactive (IR) cells in the dorsal horn of the injured side. Furthermore, although pJNK colocalizes with the astroglial marker GFAP, pJNK is only expressed in a portion of spinal astrocytes. However, pJNK occurs in spinal neurons after traumatic spinal cord injury, a condition that produces robust neuronal apoptosis in the spinal cord. JNK plays a role in stress-induced apoptosis in the nervous system.

Basic fibroblast growth factor (bFGF) is a pleiotropic cytokine synthesized and secreted by astrocytes and DRG primary sensory neurons. It strongly induces astrocyte mitosis, growth, differentiation and gliosis. bFGF is induced in the CNS in many injury conditions. After spinal cord injury, bFGF upregulates in the spinal cord, which promotes functional recovery. The release of bFGF from astrocytes probably acts in an autocrine manner to further augment astroglial activation (e.g. astrogliosis and proliferation) (Fig. 2). Because bFGF is a primary ‘activator’ of astrocytes and JNK is an important signaling molecule in spinal astrocytes, it is reasonable to ask whether bFGF activates JNK in spinal astrocytes. Intrathecal infusion of bFGF induces a marked activation of JNK in the spinal cord. bFGF also induces marked acti-
Fig. 2. Activation and response of astrocytes in neuropathic pain. Nerve injury upregulates several receptors, such as the chemokine receptors CX3CR1, P2X4 and CCR2, ETB, NKR, and NMDA in spinal astrocytes, and it increases markers such as GFAP, vimentin and S-100β. Also, nerve injury initiates a persistent downregulation of the glutamate transporters, such as GLT-1 and GLAST. Astrocytes respond to ongoing synaptic activity by mobilizing internal Ca^{++}, and this in turn leads the release of glutamate, ATP, TNF-α, IL-1β, IL-6, NO, and PGE\(_2\). AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid, ATP: adenosine triphosphate, bFGF: basic fibroblast growth factor, CCL2: cysteine chemokine ligand 2, CCR2: chemokinreceptor 2, ERK: extracellular signal regulated kinase, ET-1: endothelin-1, ET\(_B\): endothelin receptor B, FRK: fractalkine, GFAP: glial fibrillary acidic protein, GLAST: glutamate aspartate transporter, GLT: glutamate transporter, HSP: heat shock protein, IFMs: inflammatory mediators, IL-1β: interleukin-1beta, IL-6: interleukin-6, IL-18: interleukin-18, JNK: c-Jun N-terminal kinase, LPS: lipopolysaccharide, MAPK: mitogen activated protein kinase, mGluR: metabotropig glutamate receptor, NF-κB: nuclear factor-κB, NKR: neurokinin receptor, NMDA: N-terminal-D-aspartate, NO: nitric oxide, p-c-Jun: phosphorylated c-Jun, pERK: phosphorylated extracellular signal regulated kinase, PGE\(_2\): prostaglandin E\(_2\), p-JNK: phosphorylated c-Jun N-terminal kinase, PLA2: phospholipase A\(_2\), TLR: toll-like receptor, TNF-α: tumor necrosis factor alpha (dot lines are inhibition).

Activation of ERK/MAPK in astrocytes, which is evident in the spinal cord at late times following nerve injury.\(^{30}\) Meanwhile, JNK activation is likely to regulate gene transcription in spinal astrocytes via activation of the transcription factor c-Jun and other transcription factors such as ATF-2.

IL-18 and IL-18R was expressed in p-p38-IR cells of the dorsal horn at day 7 after nerve injury, which means that nerve injury increases the expression of IL-18 and IL-18R in activated microglia and astrocytes, respectively through a TLR4/p38 MAPK pathway.\(^{37}\) IL-18 is a member of the IL-1 family; IL-1β and IL-18 are related closely, and both require intracellular cysteine protease caspase-1 for biological activity. Also, nerve injury induces NF-κB activation in spinal astrocytes via the IL-18R, and the activation of the IL-18R/NF-κB signaling cascade in astrocytes contributes to hypersensitivity.

**PART II: CLINICAL APPLICATION**

1. Therapy

The past quarter century has witnessed an explosion in the understanding of pain, characterized by the elucidation of anatomic pathways, and the identification of receptors, ion channels and neurotransmitters that are involved in the transmission of nociceptive information from primary sensory neurons in the DRG to the brain. Together, advances in basic and clinical sciences and the concentrated efforts of many academic and pharmaceutical research laboratories have generated a substantial scientific
evidence base for pain. However, the development of novel effective treatments for chronic pain has been disappointingly slow.

The typical pharmacotherapies for neuropathic pain of any origin are anti-depressants, anti-convulsants, membrane stabilizers, capsaicin, baclofen, clonidine and opioid analgesic medications.\(^2\) However, they are often ineffective. There is therefore a need for new therapeutic approaches. Glial cells, specifically microglia and astrocytes within the spinal cord may offer new targets. On activation through nerve injury, glia release a variety of neuroexcitatory substances that potentiate noxious signaling, as we describe above. Of these glial products, pro-inflammatory cytokines are common spinal mediators of allodynia and hyperalgesia. New drugs targeting the glia and cytokine production as well as the nerve and DRG are now under study, as is gene therapy.

The purpose of this section is to review current efforts to identify pharmacological interventions targeting non-neuronal cells. First, we will describe the three pharmacological targets; microglia, astrocytes, and anti-inflammatory cytokines. And also, we briefly review gene therapy.

2. Pharmacological target

A better understanding of the mechanisms linked to chemokine pronociceptive effects is essential for the development of new strategies to better prevent and treat chronic neuropathic pain. Neurons are not the only players that drive the establishment and maintenance of chronic pain states, particularly neuropathic pain. Thus, one strategy for treating neuropathic pain is to develop drugs that target glial modulators of neuropathic pain instead of neurons. A number of drugs have emerged that are effective in controlling glially driven exaggerated nociceptive states although they are still in experimental stages of development.

3. Targeting microglial activation

Several studies have shown that specific microglial inhibitors and/or modulators can block and/or reverse neuropathic states.\(^58-60\) The most commonly used drugs are fluorocitrate and minocycline. Interestingly, pre-emptive and curative fluorocitrate treatment, which selectively blocks astrocyte and microglia metabolism,\(^61\) inhibits neuropathic pain,\(^62-63\) whereas the antibiotic minocycline, a specific microglial inhibitor,\(^63\) blocks the development of neuropathic pain states but does not reduce pain that is already established.\(^64\) Although the animal studies mentioned above have described a broad array of drugs that inactivate or disrupt glial function, most of them are not appropriate for human application. For example, fluorocitrate, although highly effective at blocking the onset of neuropathic pain, blocks glial uptake of excitatory amino acids and has a narrow dose range, with higher doses becoming neurotoxic.\(^64\) However, minocycline is relatively safe and tolerable, and it has undergone various clinical trials. Minocycline inhibits not only the spinal p38 signaling cascade but also the release of pro-inflammatory mediators. It reduces oxidative stress in a neuropathic pain condition,\(^65\) and also reduces naloxone-precipitated withdrawal as ibudilast does. Co-administration of minocycline and ibudilast with morphine or oxycodone results in a three-to five fold increase in acute analgesic potency and opioid withdrawal due to the suppression of glial pro-inflammatory responses.\(^66\) Propentofylline is a glial modifying drug and a methylxanthine derivative. Given within 6 weeks after nerve injury, it can reverse neuropathic pain by inhibiting microglial responses and suppresses astrocyte activation, and it controls pain behavior in rodent models of enhanced pain states (Fig. 3).\(^67\)

Daily intrathecal administration of the p38 signaling cascade inhibitor SB203580 prevents spinal nerve ligation induced mechanical allodynia, but it cannot reverse neuropathic pain symptoms when given after nerve injury.\(^68\) These observations suggest that the p38 signaling cascade and microglia are only important for the induction or development of neuropathic pain, and a p38 or microglia inhibitor can only treat neuropathic pain at early stages. If true, this limits the therapeutic potential of p38 and microglia inhibitors. Moreover, p38 inhibitors FR167653 or CNI-1493 also prevent the development neuropathic pain symptoms in the spared nerve injury model and in a sciatic inflammatory neuropathy model (Fig. 3).\(^69,70\) The CD40-CD154 and P2Y12 inhibitor, suramin, decreases the release of inflammatory cytokines such as IFN-\(\gamma\), IL-6, and IL-8. It also prevents the CD154-induced proliferation of human

B cells. However, its clinical usefulness in the management of AIDS and cancer is limited because of its immuno-suppressive action.71)

Current p38 signaling cascade inhibitors are not isoform-specific due to their design to target common-ATP binding sites on the kinases and the structural similarities between the p38 isoforms. To determine the distinct role of p38 isoforms in the development of neuropathic pain, it is important to specifically inhibit a particular isoform. More importantly, isoform-specific inhibition will minimize the side effects produced by blocking all p38 isoforms with a general inhibitor. A major concern is that p38 signaling cascade inhibitors may produce psychiatric and cardiovascular side effects or liver toxicity.72) A pressing need exists for the development of more specific p38 signaling cascade inhibitors, such as activation status-specific inhibitors and isoform-specific inhibitors. Local delivery of p38 signaling cascade inhibitors or an siRNA strategy also may merit consideration. A combination of MEK and p38 signaling cascade inhibitors suppresses endotoxin-induced expression of iNOS and TNFα in microglia with a much greater efficacy than each inhibitor alone.73) Several MAPK inhibitors including SB281838, BIRB0796, Ro320-1195, and SCIO-469 have undergone testing in clinical trials for inflammatory diseases such as rheumatoid arthritis as well as for cancer.4,74) Spinal inhibition of the late-phase activation of ERK by intrathecal MEK inhibitor reverses mechanical allodynia, supporting a role of astrocytic ERK.
in the maintenance of neuropathic pain (Fig. 3). The quinidine homodimer Q2 reverses the P-glycoprotein, an ATP-dependent drug efflux pump, mediated paclitaxel resistance phenotype as well as inhibits verapamil-stimulated ATPase activity.

The chemokine CCL2 is released in an activity dependent manner from the central terminals of primary sensory neurons in neuropathic pain conditions. Thacker et al. reported that intraspinal CCL2 activates spinal microglia, and this is inhibited by a specific neutralizing antibody to CCL2. The selective CCR2 antagonist (INCB3344) completely prevents the pronociceptive effects of CCL2 via direct spinal activation of CCR2. Jung et al. suggested that the antagonism of MCP-1/CCR2 signaling may offer a novel therapeutic approach in treating neuropathic pain (Fig. 3).

TLR4 is a key microglial receptor in the initiation of behavioral hypersensitivity after peripheral nerve injury. TLR4-knockout and point mutant mice develop less neuropathic pain and these animals show reduced glial activation and strongly decreased expression of pain related cytokines. Therefore TLR4 receptors could be a novel and intriguing target for neuropathic pain control. The TLR4 antagonists (+)-naloxone and (-)-naloxone, also opioid antagonists, can each fully reverse established neuropathic pain with multi-day administration. New TLR4 antagonists (FP-1) that inhibit NF-κB activation can counteract well-established neuropathic pain induced in mice by chronic constriction injury of the sciatic nerve (Fig. 3).

4. Targeting the astrocyte activation

The JNK signaling cascade is localized in spinal astrocytes after nerve injury. A peptide inhibitor of JNK, derived from the JNK-binding domain of JNK-interacting protein-1 (JIP-1), can block selectively the access of JNK to c-Jun and other substrates by a competitive mechanism. A convert to D-form amino acids further makes the peptide proteinase-resistant. This highly-specific peptide inhibitor, called D-JNK1-1 is a potent neuroprotectant against excitotoxicity of cortical neurons. Spinal infusion of this inhibitor intrathecally does not change basal pain thresholds, but it prevents mechanical allodynia. Infusing D-JNK1-1 intrathecally via osmotic pump effectively reverses spinal nerve ligation-induced allodynia for several days. A single bolus injection of D-JNK1-1 inhibitors also effectively reverses mechanical allodynia for over 12 hours. D-JNK1-1 is more potent than the small molecule inhibitor SP600125. D-JNK1-1 also suppresses nerve injury-induced activation of c-Jun in astrocytes, a major downstream target of JNK.

Falsig et al. have shown that mixed linkage kinase (MLK) inhibitors such as CEP-1347 are potent astrocyte immune modulators. In astrocytes in culture, CEP-1347 blocks activation of JNK, expression of COX-2 and iNOS, and release of nitric oxide, PGE2 and IL-6 following challenge with a mixture of cytokines. A neutralizing antibody to bFGF reduces SNL-induced expression of GFAP in the spinal cord and reverses SNL-induced tactile allodynia, which indicates that endogenous bFGF contributes to maintaining neuropathic tactile allodynia (Fig. 3).

5. Targeting the anti-inflammatory cytokines

Research targeting anti-inflammatory glial activation processes, such as cytokine production and/or regulation, for therapeutic purposes is an alternative line to yield some interesting results. Cannabinoid receptors, both type 1 (CB1) and type 2 (CB2), are potential therapeutic targets for inflammatory neurodegenerative diseases and neuropathic pain. Activation of CB2 receptors has beneficial effects in animal models of neuropathic pain. However, one study identified CB2 expression mainly on neurons after chronic neuropathic conditions. The discrepancies between studies may reflect differences in the specificities of the antibodies used for immunohistochemical detection. Despite these differences, several studies report that activation of the cannabinoid system leads to anti-inflammatory processing, such as increased expression of anti-inflammatory markers (for example, ED2 (also known as CD163)). These results suggest that facilitating anti-inflammatory aspects of glial activation is a more powerful approach to controlling noxious signaling than simply preventing pro-inflammatory glial activation. Thus, enhancing anti-inflammatory cytokines, such as IL-10, could be a more beneficial approach than administering antagonists of the proinflammatory cytokines themselves or blocking...
the normal function of glia by globally preventing glial activation. However, Gunnarsson et al.\textsuperscript{83} reported that systemic lupus erythematosus like symptoms was observed with the patients increased serum IL-10 levels. A major challenge facing new drug-development strategies for the treatment of neuropathic pain is targeting the pathological actions of proinflammatory cytokines and chemokines from astrocytes and microglia in the spinal cord without altering their protective and recuperative roles.

6. Gene therapy

Most pharmacologic treatments for neuropathic pain have limited effectiveness or produce undesirable side effects. Intrathecal delivery of conventional analgesic medications can enhance the pain-relieving potency of drugs by physically directing the drug in highest concentration to the spinal cord. Here, the first synapse in the pain pathway between nociceptive neurons terminals of the dorsal root ganglion (DRG) and second order neurons in the spinal cord provide an attractive target to modulate nociceptive neurotransmission. Multiple methods can transfer genes to the CNS for therapeutic purposes, including the transplantation of engineered cells expressing the therapeutic gene into the CNS, transgene injection by intramuscular or intraneural injection, vector injection directly into the brain or spinal cord, and silencing endogenous gene expression through nucleotide based (DNA or RNA) methods.

Research on therapeutic gene transfer to the CNS for the treatment of chronic neuropathic pain and other pathological pain syndromes has been underway for only about a decade. The optimal treatment strategy is still uncertain. Both viral and non-viral methods have successfully treated chronic neuropathic pain in rodent models.\textsuperscript{84,85} Plasmid-based gene delivery is attractive because it is easy, inexpensive, and lacks the dangers associated with some viral vectors, such as insertional mutagenesis that can result in tumors.

The use of gene transfer, in place of drug delivery to achieve the continuous release of short-lived bioactive peptides in or near the spinal dorsal horn underlies the most common strategies for gene therapy of pain. There are two principal models. The first involves intrathecal injection of vectors derived from adenovirus, aden-associated virus or lipid encapsulated plasmids. In the second approach, neurons of the DRG are transduced by injection of herpes simplex virus-based vectors in to the skin.

Although small molecules offer promising approaches to treat neuropathic pain, recent studies using gene-transfer techniques offer a unique advantage: targeted drug delivery to discrete areas of the nociceptive transmission pathway. Targeted and chronic spinal transgene expression may be an important mechanism by which to achieve long-term neuropathic pain control. Gene therapy has gained momentum as a tool by which to target neurons or glia for pain control.\textsuperscript{60} Intrathecal delivery of IL-10 or IL-2 genes reversed peripheral nerve injury-induced thermal hyperalgesia and mechanical allodynia for up to 4 weeks\textsuperscript{84} while leaving normal pain thresholds intact. In these studies, viral vectors were administered by direct spinal or intrathecal injection into the cerebrospinal fluid for gene transfer to the CNS. Direct spinal gene delivery of anti-inflammatory cytokines, such as IL-10, leads to a robust and sustained reversal of neuropathic pain in rodent models. Ongoing studies show further improvement of targeted spinal cord IL-10 gene delivery using synthetic polymers that are engineered to encapsulate the transgene and release it on intrathecal injection.\textsuperscript{86} Intramuscular or intrathecal gene transfer using viral vectors encoding IL-4 or IL-10, respectively, reversed neuropathic pain for 4 months.\textsuperscript{84} Anti-inflammatory transgenes delivered by viral methods include ones encoding IL-4 and the soluble TNF receptor that binds TNF-\textalpha, thereby terminating its action.\textsuperscript{85}

Recent reports identify more obstacles to overcome before clinical application, including two fatal incidents associated with viral vector usage. The main problems are: 1) viral vectors used as carriers for gene therapy have failed in safety trials on two occasions; and 2) the human immune response and the oncogenic property of the vectors have restricted the advancement of gene therapy as a therapeutic tool.\textsuperscript{87} Although gene transfer for the treatment of pain is slowly moving towards the clinic, chronic pain represents an urgent clinical problem for which there is a substantial unmet need. This report provides additional hope for the future in this regard. In the near future, gene
therapy may provide one or more novel options for physicians who struggle to treat patients suffering from intractable pain. For patients who fail interventional therapies or intrathecally administered drugs, gene therapy may promise improved pain control. Currently, gene therapy is primarily experimental, with different vectors and techniques competing for clinical application.

Therapeutic strategies attempting to control neuropathic pain states by targeting glial function are attracting much attention and are beginning to yield promising results. So does gene therapy. The drugs under study fall into three major categories: 1) drugs that attenuate microglia and/or astrocyte activation; 2) drugs that inhibit pro-inflammatory cytokine production; and 3) anti-inflammatory drugs.

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