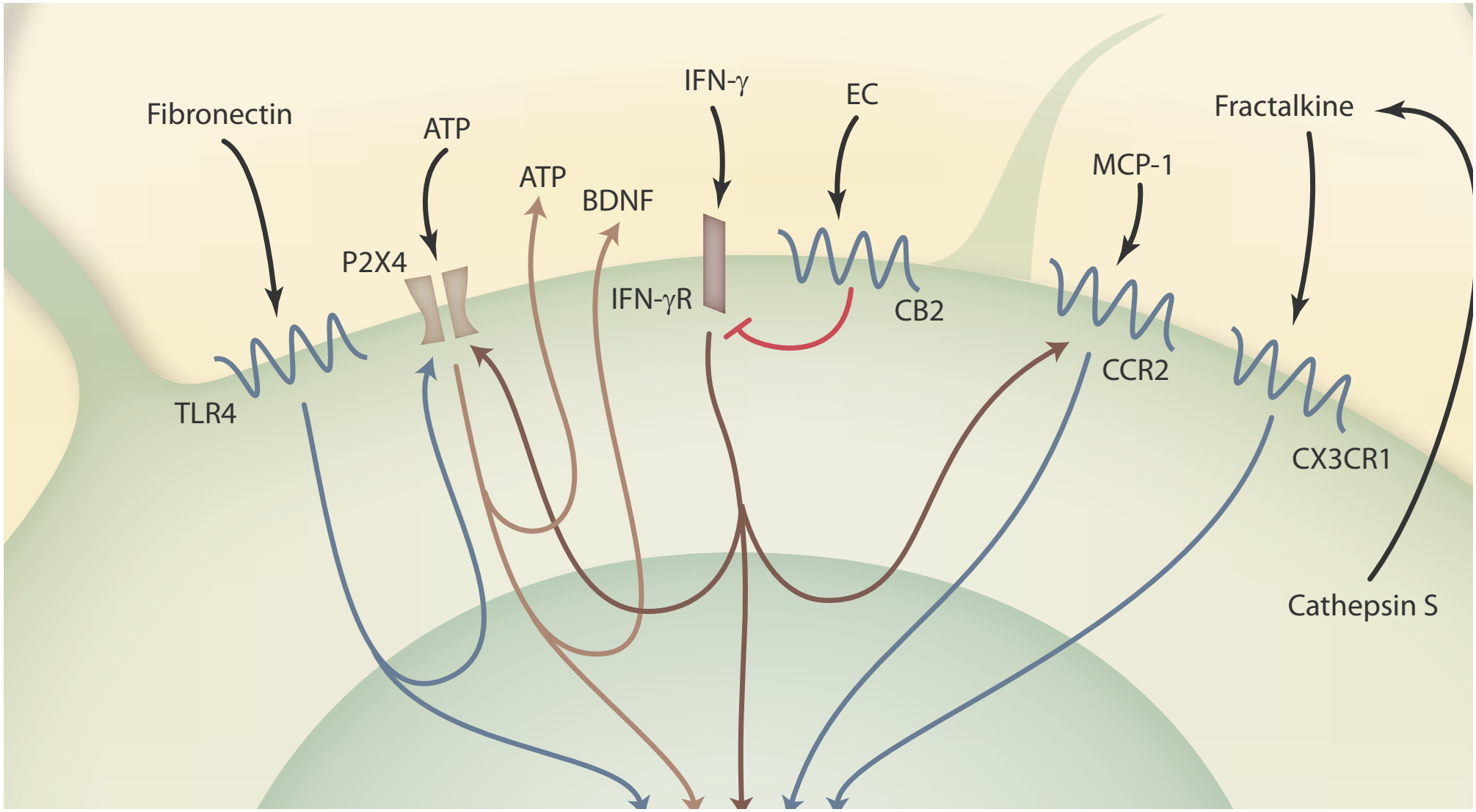
MICROGLIAL INVOLVEMENT IN NEUROPATHIC PAIN

Five mechanisms as focal points for assessing opportunities for the development of neuropathic pain therapies.



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INTRODUCTION

Acute nociceptive pain resulting from nerve damage incurred because of trauma, surgery, infection, or disease (i.e., diabetic neuropathy, cancer, autoimmune disease), is typically followed by a period of recovery in which pain and inflammation subside and normal functionality is restored. However, 7% to 18% of patients instead develop neuropathic pain (NP), or pain that becomes chronic and debilitating, characterized by allodynia (pain caused by normally non-painful stimuli), hyperalgesia (heightened response to painful stimuli), and spontaneous pain that often leads to behavioral disabilities including insomnia and depression. Unfortunately, NP is extremely challenging to treat and many patients find that their symptoms are not ameliorated by currently available pharmacotherapies such as non-steroidal anti-inflammatories and opioids. [1-5]

Early investigations into the pathophysiology of NP were focused primarily on the effects of peripheral nerve injury on neurons in the pain processing pathway. These include, for example, modifications in expression patterns of receptors, ion channels, and neurotransmitters, alterations in synaptic connectivity, and cell death. The collective result is increased sensitization and excitability of neurons causing exaggerated responses to noxious as well as innocuous stimuli. While these changes in the physiology of the neural pain processing pathway are certainly important to the etiology of NP, esearchers now agree that there is more to the story. [1-3] The observation that neuroinflammation seems to be a common thread in NP, regardless of the conditions under which NP develops, opens up a whole new avenue for investigations into NP pathology. [1,2,4] Since the primary cell type responsible for immune-like functions in the CNS is microglia, many researchers have turned their attentions toward working to better understand microglial physiology and its potential involvement in NP. [1-3]

Microglial Cells

Of the roughly 70% of cells in the CNS that are glia, approximately 5% to 10% are microglial cells. Microglial cells are derived from peripheral myeloid progenitor cells that enter the CNS during embryonic development. Though ubiquitous in the CNS, microglial cell densities vary by region. They function to provide structural and trophic support to neurons and serve as the resident immune-competent cells of the CNS, tasked with the detection of infections and injuries, protection of healthy tissues, elimination of disturbances, and restoration of homeostatic conditions. Normally, microglial morphology is characterized by a small soma with many thin, branched processes. Microglial processes come in contact with neurons, endothelial cells, and astrocytes, but not other microglial cells. In fact, each cell appears to be responsible for a distinct territory, within which it constantly samples the extracellular microenvironment by sweeping its processes through the tissue without disrupting neuronal connectivity. [1-8]

Microglial cells have a very low threshold for activation and can be activated by a wide variety of stimuli. Once activated, they undergo morphological and physiological changes and they mobilize and proliferate. Activated cells display enlarged soma with shorter processes or even amoeba-like shapes and dramatically altered gene expression profiles. They home to injured areas, perform phagocytic and antigen presentation functions, and re-enter the cell cycle to increase their number. As microglial cells are not electrically coupled with other cells, they act solely via the release of diffusible mediators to communicate with neighboring cells in a paracrine fashion. [1-7] Microglial phenotypes are extremely plastic. The process of microglial activation is neither an "all-or-none" commitment, nor a linear path, which allows for creation of a wide range of activated phenotypes to achieve very graded responses to real or perceived threats to the CNS. Taken together with evidence of microglial populations having already "built-in" heterogeneity and the possibility that when individual cells are activated once, they may respond differently when activated again through potentially long-lasting epigenetic mechanisms, the picture of microglial activities in the CNS becomes extremely complex. [6,7] ■

Involvement in Neuropathic Pain: 5 activation pathways

While microglial participation in NP pathophysiology has been investigated using a wide variety of experimental rodent models, some of the more popular include the chronic constriction injury (CCI) model in which the sciatic nerve is loosely ligated, the spinal nerve ligation (SNL) model in which either the L5 or L6 spinal nerves are tightly ligated, and the streptazocin-induced diabetic neuropathy (STZ) model in which pancreatic β-cells are selectively killed. [1,9] Several lines of evidence compiled using these models have demonstrated the intimate involvement of microglial cells in the establishment of NP. [1-4,7,8] More specifically, the process of microglial activation is now thought to be both necessary and sufficient for NP initiation. [8] Although there is some variability between results obtained using the different NP models, generally microglial cells in the ipsilateral dorsal horn of the spinal cord become activated within approximately 4 hours, increase 2 to 4 fold in number by 2 days, and remain active for several months after peripheral nerve injury. [1,3-5,7] These effects can be suppressed by non-specific microglial inhibitors, such as the antibiotic Minocycline, in animal models of NP including CCI, SNL, STZ, and others. [1-3,8,10] Although in the context of NP, local, responding microglial cells are known to be activated by a broad range of stimuli, 5 predominant activation pathways appear to be most critical and are identified here by their major ligand or receptor. [1-5,7,8]

TLR4

In the context of NP, toll-like receptor family member 4 (TLR4) is known to be expressed exclusively on spinal microglia and significantly up-regulated upon peripheral nerve injury. TLR4-knockout mice display reduced effects of CCI-induced nerve damage. Similarly, TLR4 loss-of-function mutant mice as well as TLR4 antisense oligonucleotide-treated rats both display attenuated NP symptoms after nerve damage. Further, intrathecal administration of a TLR4 antagonist after CCI treatment results in relief of NP symptoms. Many exogenous and endogenous ligands are known to stimulate TLR4-mediated signaling. However, both in vitro and in vivo studies involving SNL-treated

animals implicate Fibronectin in NP-related TLR4 signaling. Fibronectin is an extracellular matrix protein that is commonly produced in response to tissue injury. When administered intrathecally to intact rats, Fibronectin induces microglial up-regulation of the purinergic receptor, P2X4, and symptoms of NP. This stimulation of P2X4 expression can be suppressed by interruption of Fibronectin binding the TLR4 receptor after SNL injury in rats. [2,3,7,8]

P2X4

Injured afferent neurons and neighboring astrocytes spill ATP into the extracellular space causing the activation, migration, and proliferation of nearby microglial cells via the purinergic, ligand-gated, cation channel, P2X4. Using SNL-treated animals, researchers have shown that P2X4 is expressed exclusively in spinal microglial cells, that its expression is up-regulated after nerve damage, and that the inhibition of P2X4 up-regulation prevents NP symptoms from developing. Pharmacological blockade of P2X4 ameliorates NP symptoms induced by SNL, and mice lacking functional P2X4 fail to develop NP symptoms after SNL injury. Intrathecal delivery of either ATP or cultured, P2X4-stimulated, microglial cells can induce NP in intact rats. In addition to promoting the activation, mobilization, and proliferation of microglial cells, P2X4 signaling is known to cause microglia to release additional ATP as well as the neurotrophic factor, BDNF. In the context of NP, ex vivo studies suggest that BDNF causes an inversion of the polarity of inhibitory currents in spinal tissue resulting in the hyperexcitability of dorsal horn neurons. [1-4,7,8]

IFN-γ & CB2

Interferon gamma (IFN-γ) is released from neurons after peripheral nerve injury and the IFN-γ receptor (IFN-γ R) is constitutively expressed on microglial cell surfaces, thus suggesting a potential mechanism for the stimulation of microglial activation in the context of NP. Intrathecal administration of IFN-γ is known to induce NP in intact mice, an effect that can be suppressed by the well-known inhibitor of microglial cell activation, Minocycline. Additionally, IFN-γ knockout mice and IFN-γ R knockout mice subjected to SNL or other means of peripheral nerve injury exhibit reduced microglial activation and number in the ipsilateral dorsal horn and attenuated NP symptoms. IFN-γ signaling in microglial cells is known to up-regulate expression of a number of genes including P2X4 and the chemokine receptor, CCR2. [1,7,8] Although

the exact mechanism is not yet known, interference with IFN-γ signaling, and therefore prevention of microglial activation, can be achieved by endocannabinoids, which are known to be released from both neurons and microglia under NP conditions. While the endocannabinoid receptor CB1 is widely expressed in CNS tissues, the CB2 is predominantly expressed on immune and immune-like cells including spinal microglia. Microglial CB2 expression is up-regulated after peripheral nerve injury in a variety of NP animal models. Over-expression of CB2 or application of CB2 agonists reduces microglial activation and NP symptoms while treatment with CB2 antagonists blocks these effects in multiple NP models. [1,3,11] Further, systemic administration of CB2 agonists prevents microglial activation and alleviates NP symptoms in STZ rats. [11]

MCP-1

Monocyte chemoattractant protein 1 (MCP-1, a.k.a. CCL2) has been shown to be up-regulated in afferent sensory neurons and ipsilateral dorsal horn tissue of CCI-treated rodents, results which have been replicated using many of the NP models. Further, evidence from SNL-treated rodents suggests MCP-1 is also released from spinal astrocytes in response to injury. Interestingly, it appears that elevated MCP-1 release is caused by increased electrical activity observed after peripheral nerve injury and mirrors both the spatial and temporal pattern of subsequent microglial activation after CCI. Intrathecal administration of MCP-1 to intact animals induces microglial proliferation and NP symptoms while similar delivery of an anti-MCP-1 neutralizing antibody attenuates microglial activation. Further evidence for the importance of MCP-1 in the development of NP comes from work on the MCP-1 receptor, CCR2. CCR2 expression has been documented on TRPV1-expressing sensory neurons, known for their involvement in the pain processing pathway, and on spinal microglia. Mice lacking the CCR2 gene fail to develop NP symptoms after peripheral nerve injury. Further, intrathecal injections of a CCR2 antagonist completely abrogate NP symptoms induced in either MCP-1-treated intact animals or CCI-treated animals. [1-4,7,8]

Fractalkine

Fracktalkine (a.k.a. CX3CL1) is highly-expressed in its membrane-bound form on the surface of CNS neurons. It is shed from neurons by Cathepsin S thereby generating its soluble form, which

binds its only receptor, CX3CR1, expressed on the surface of microglia. In vitro, ex vivo, and in vivo studies all implicate this ligand/receptor pair as playing an important role in NP initiation. In vitro, primary neuronal cells receiving excitotoxic stimuli rapidly shed Fractalkine from their surfaces, which results in the homing of primary microglial cells. These data are confirmed and extended by ex vivo studies, which additionally show that the proteolytic cleavage of Fractalkine from neuronal membranes is achieved by Cathepsin S secreted from activated microglial cells. After peripheral nerve injury in vivo, both Fractalkine and Cathepsin S levels are elevated in CSF. Further, in both CCI- and SNL-injured animals, CX3CR1 expression is up-regulated in microglia. Finally, exogenous Fractalkine administration induces NP symptoms while delivery of anti-CX3CR1 neutralizing antibody attenuates symptoms in CCI-treated rats. [1-3,7,8]

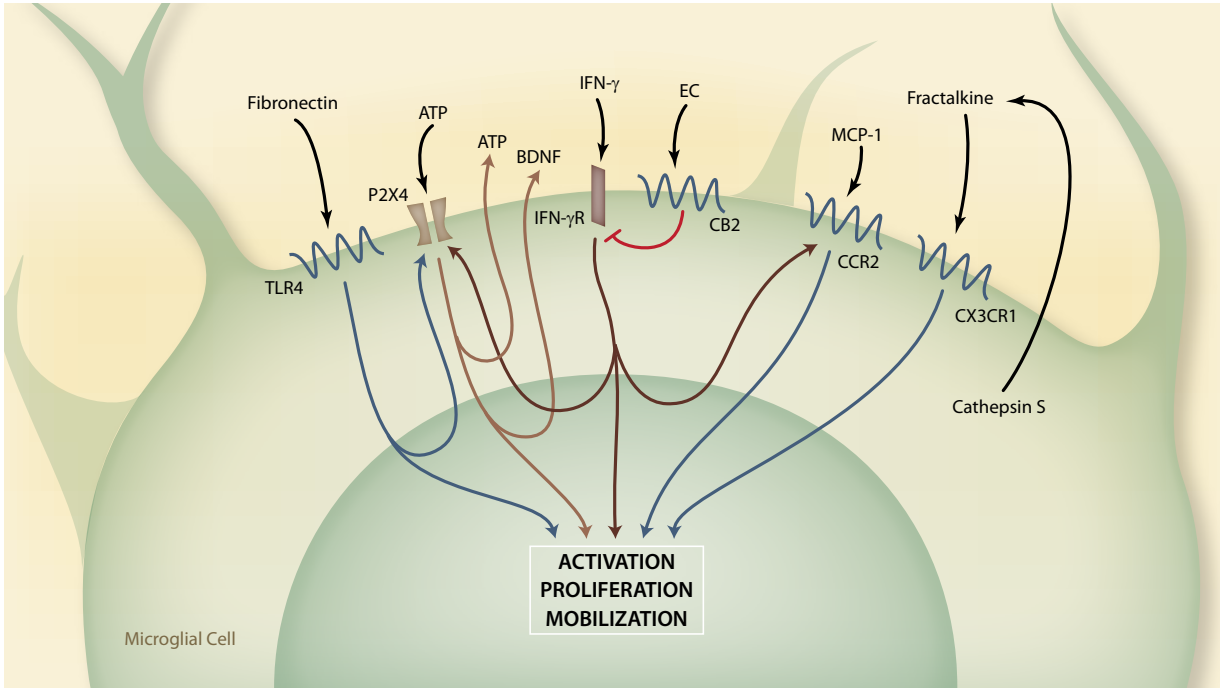

Figure 1 presents a simplified overview of the 5 key pathways to microglial activation described.

Conclusion

These mechanisms have emerged as exciting new focal points for assessing opportunities for the future development of pharmacotherapies, gene therapies, or cell-based therapies for NP patients. However, the path to clinical application of these ideas is not without its pitfalls. For example, many of the compounds employed to disrupt microglial function in animal studies are not suitable for use in humans. Further, several of the drugs that target pro-inflammatory cytokines known to be released by activated microglia are unable to cross the blood-brain barrier. Finally, microglial cells have a number of very important "housekeeping" functions including providing nutritive and structural support to neurons, and microglial activation is not always bad as it initiates protective and reparative functions as well. Perhaps the key to prevention or elimination of NP is in the promotion of well-controlled microglial responses that lean more toward the neuroprotective effects and away from the neurotoxic effects of microglial activation. ■

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ANIMAL MODELS OF PAIN:

NOCICEPTIVE PAIN

Inducer	Species	Mediating Factors
Capsaicin	Rats, mice	VR1
Tail Flick	Rats, mice	external stimuli
Visceral	Mice	Acid

INFLAMMATORY & ARTHRITIC PAIN

Inducer	Species	Mediating Factors
Carrageenan	Rats	PGE2 and Mast cells
CFA (poly-RA)	Rats	Cyokines, PG, Macrophages, Neutrophils
CFA (mono-RA)	Rats, mice	Cyokines, PG, Macrophages, Neutrophils
Collagen (RA)	Rats	Cytokine, Macrophages, Tcells
Adjuvant (RA)	Rats	Cyokines, PG, Macrophages, Neutrophils
MIA (OA)	Rats	

NEUROPATHIC PAIN

Inducer	Species	Mediating Factors
Surgery of sciatic nerve (CCI)	Rats	Inflammation of the nerve
Surgery of sciatic nerve, cutting (SNL)	Rats, mice	Damage to the nerve
STZ (diabetic)	Rats	Damage to nerve ending due to hyperlgycemia and hypoxia
Taxol	Rats	Taxol mediated neurotoxicity by interfering with sensory neuron skeleton structure

POST-OPERATIVE PAIN

Possible Inducer	Species	Mediating Factors
Incisional Pain (Brennan)	Rats	Inflammation, wound
Incisional Pain	Pigs	Inflammation, wound

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