Neuroimmune Activation and Chronic Pain

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Chronic pain is an extremely debilitating disease syndrome for which current treatment modalities are largely ineffective. This article presents the recently proposed contributions of neuroimmune activation to the maintenance of chronic pain. The theory of neuroimmune activation postulates a pathway that links peripheral neuronal injury/inflammation with the activation of central nervous system neuroglial cells, which contributes to sustained neuronal hyperexcitability. Literature generated by the emerging field of central nervous system glial cell research, including genetic therapies, was reviewed to provide empirical support for this pathway. The clinical implications of neuroimmune activation to improved treatment of chronic pain states are discussed.

Keywords: Chronic pain, neuroglia, neuroimmune activation, neuroinflammation.

Chronic pain syndromes affect many people in the United States and the world[1] and are responsible for tremendous suffering and disability. The scope and cost of this problem are staggering. Approximately 15% to 25% of adult American workers suffer from back pain, with an annual cost to the American workforce of $7.4 billion[2]; 14.7% of workers have arthritis-related pain, with a cost of $7.1 billion[3]; and 2 to 4 million workers have diabetes-related peripheral neuropathic pain, with a cost of $3.65 billion.[4-6] Yet there is a lack of effective treatment modalities for the chronic pain disease process; despite multiple pharmacologic and nonpharmacologic therapies, patients continue to suffer. Faced with such a treatment conundrum, clinicians and researchers have been actively searching for new explanations for the intransigence of chronic pain syndromes. In this article, recent information is reviewed, which examines neural-immune relationships and the pathophysiology of chronic pain. Beginning with normal nociception, connections between peripheral nerve damage and inflammation and proinflammatory changes in the central nervous system (CNS) will be developed into an explanatory theory, along with recommendations for future therapeutic interventions.

Chronic Pain and Neuroimmune Activation: The Theory

In an effort to address the lack of effective treatment options for chronic pain, Linda Watkins and Steven Maier of the Psychology Department, University of Colorado in Boulder, began examining links between *peripheral* events and *central* nervous system inflammatory changes, by investigating the phenomenon of infection-associated hyperalgesia.[7,8] In extensive preclinical studies of the phenomenon (Figure 1), a pathway linking peripheral infection with CNS neuroglial cell activation was identified.[8] Vagal receptors are stimulated by proinflammatory molecules produced by innate immune cells responding to microbial invasion; the resulting vagal impulses activate the medullary nucleus tractus solitarius, sending neural signals superior to the cortex and inferior to the spinal cord. These peripheral neural signals activate the neuroglial cells (astrocytes, microglia, oligodendrocytes, and others), immunocompetent innate cells indigenous to the CNS. Neuroglia provide immunosurveillance and protection, and astrocytes regulate growth factors in the synapses. Neuroglial activation by vagal neural signals stimulates production of proinflammatory cytokines; in the cerebral cortex, behaviors such as social withdrawal, tiredness, and poor appetite are elicited. In the spinal cord, microglial proinflammatory cytokine (PIC) production is accompanied by hyperalgesia, presumably caused by the effect of PICs on neuronal cytokine receptors, which influence neuronal hyperexcitability (explained in the next paragraph).

These findings led multiple investigators[1,7,9] to speculate that the barrage of afferent sensory nerve impulses arising from *peripheral* nerve damage, trauma, or inflammation could likewise activate *central* nervous system microglial cells. Based on accumulating evidence, discussed in the next section, the following theorized sequence of events is now widely accepted. When peripheral nerve damage (from trauma or inflammation) and prolonged pain occur, the immune-mediated inflammatory events along the nerve cause proinflammatory mediator molecules (PMMs) such as glutamate and substance P to be synthesized by and transported through the axon to the dorsal horn[7] (Figure 2). The afferent neuron begins to release molecules of fractalkine, an inflammatory chemoattractant (a cytokine that attracts leukocytes) from the neuronal surface at the synapse in the dorsal horn of the spinal cord. Fractalkine, glutamate, and substance P molecules diffuse to the microglial cells, which are activated first to begin producing proinflammatory cytokine molecules and other PMMs (Table), which in turn activate the astrocyte cells. The glial-produced PMMs diffuse to the pain nerves in the spinal cord and

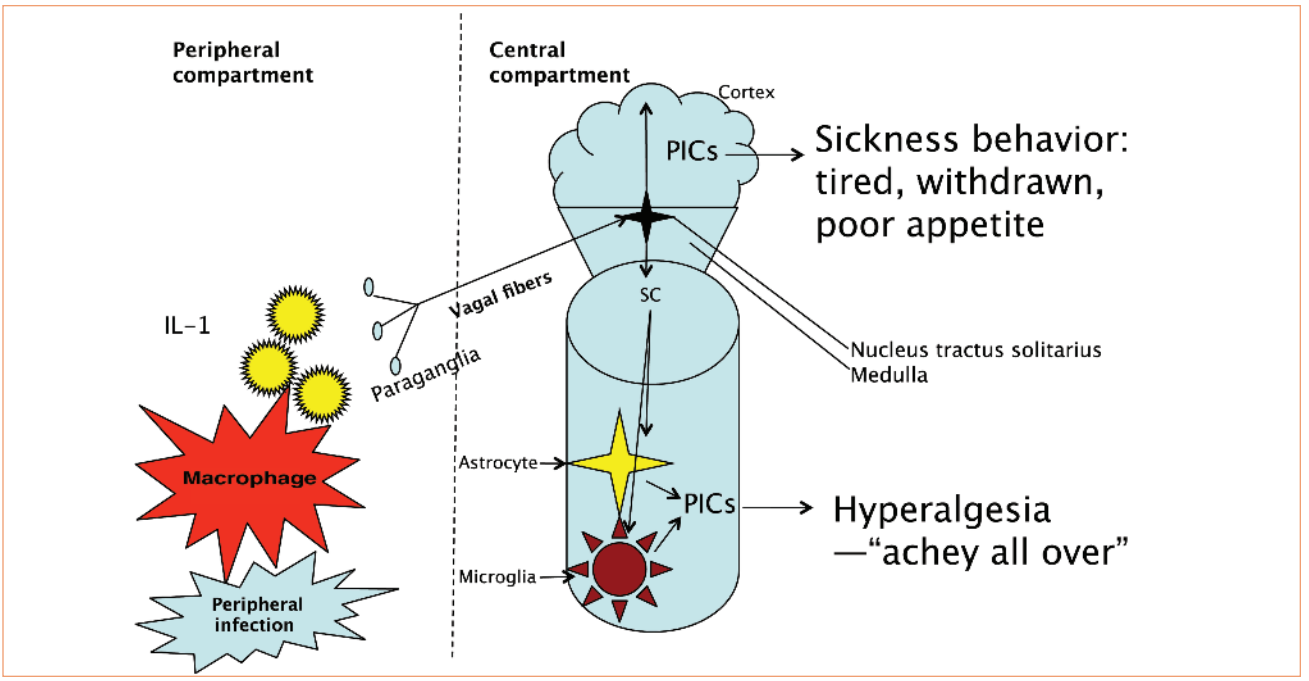

Figure 1. Link Between Peripheral Infection and Centrally Mediated Hyperalgesia

Macrophage activation by a peripheral infection leads to release of interleukin-1 (IL-1), stimulating paraganglia sensory endings of tissue vagal fibers. Vagal impulses are conducted to the medullary nucleus tractus solitarius. When activated, this vagal center sends impulses rostrally to the cortex, causing proinflammatory cytokine release, driving "sickness behavior"—tiredness, decreased socialization, and poor appetite. Impulses from the nucleus tractus solitarius spreading caudally produce proinflammatory cytokine (PIC) release in the spinal cord (SC), associated with the hyperalgesic "achiness" of illness. These findings by Watkins et al[7] led multiple investigators to speculate that peripheral neural events might influence central immune cells and somehow produce hyperalgesia.

activate receptors specific to each molecule on the neuronal surface. This produces a cascade of intracellular signals, resulting in the opening of neural sodium and calcium (cation) channels. As the cation channels open, the ascending pain nerves become progressively depolarized or hyperexcited, firing more frequently, prolonging and intensifying the pain experience (Figure 2). A positive feedback system now exists, driven by continuing afferent peripheral nerve PMM release, glial activation and PMM release, and neuronal hyperexcitability.

Empirical Support for Neuroimmune Activation Theory

Chronic pain is theorized to be driven by not only neuronal mechanisms such as ectopic firing but also activation of neuroglia cells, producing an immune inflammatory condition in the CNS.[7] It is appropriate to discuss the origin and empirical support for this hypothesis regarding the pathophysiology of chronic pain syndromes.

- *Failure of Neuronal Therapies.* Traditional chronic pain treatment targeting pain neurons as the key to reducing or eliminating pain have been only marginally successful, leaving many patients with poorly relieved chronic pain.[7] Surgical approaches in which neurons or impinging structures such as vertebral laminae are removed or transected have an extremely variable rate of success, and often exacerbate pain states.[10] Local anesthetic drugs can stop pain impulses via sodium channel blockade; however, the molecules are not specific to nociceptor fibers.[11] Thus, pain relief is accompanied by numbness and motor weakness, rendering this modality inappropriate. A new drug, ziconotide, is more specific to nociceptive fibers, yet it must be administered intrathecally and is associated with frequent and serious side effects, limiting its usefulness.[12] Pain specialists implant neurostimulators to treat chronic neck or back pain; the pain is replaced with a tolerable paresthesia or tingling sensation.[13] Yet these stimulators require surgical implantation, with the attendant risks, and repeat surgery for battery changes, and do not work in a sizable proportion of patients.

Opioid drugs are administered orally, parenterally, and intrathecally to patients with chronic pain; however, tolerance and dependence-related side effects develop rapidly, often accompanied by diminished efficacy.[1] Evidence is accumulating that chronic opioid therapy may contribute to neuroglial activation and central inflammation.[1,7] The acute interaction of opioid ligands with the mu opioid receptor (MOR) is characterized by postsynaptic neuronal hyperpolarization related to increased potassium conductance and inhibition of nociceptive neuronal neurotransmitter release.[11] *Long-term* opioid molecule-MOR interaction, in contrast, is associated with the G-protein-mediated activation of protein

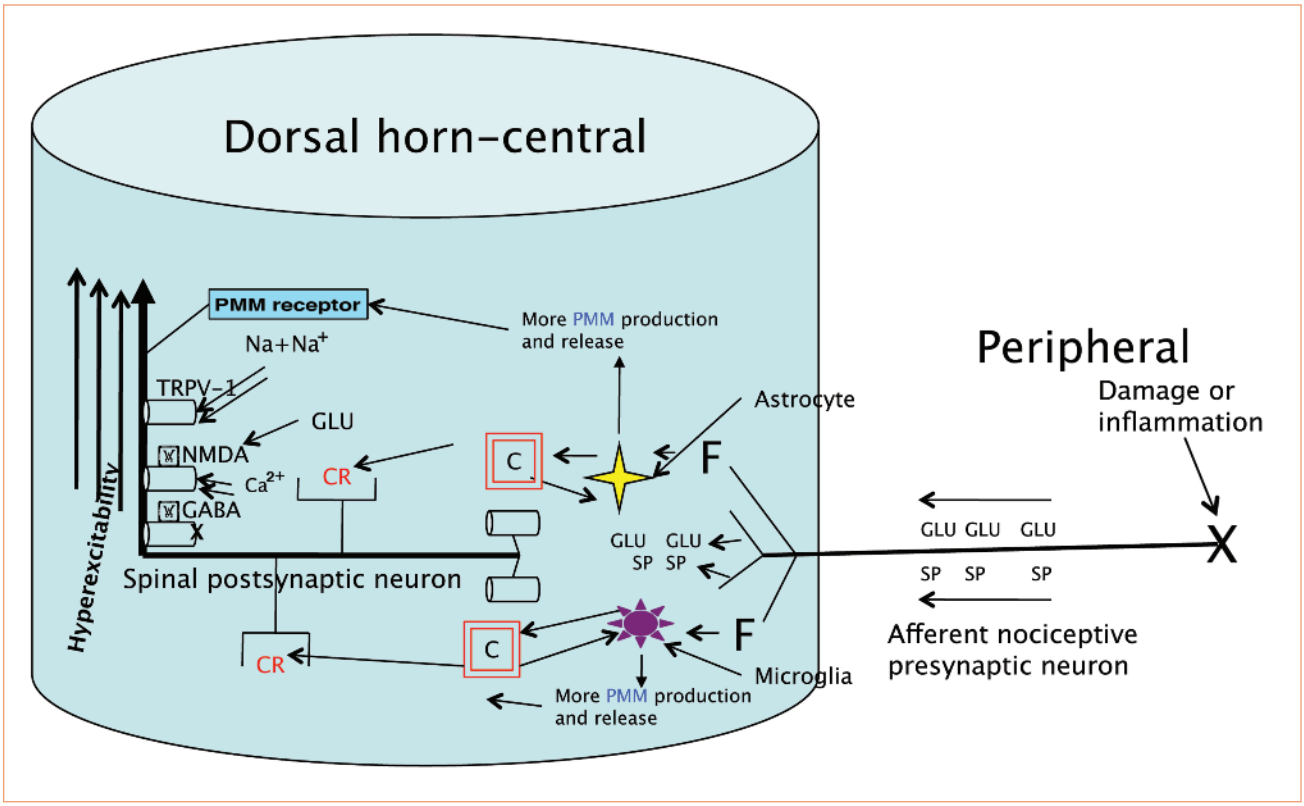

Figure 2. Peripheral Nerve Damage/Inflammation Causes Central Proinflammatory Changes That Initiate a Positive Feedback Cycle, Driving and Maintaining Chronic Pain.

Afferent nociceptive signaling causes neuronal fractalkine (F) release at the dorsal horn synapse; F molecules activate neuroglia, which produce proinflammatory cytokine molecules (C). Cytokines interact with neuronal receptors, down-regulating gamma aminobutyric acid (GABA) inhibitory receptors, and up-regulating *N*-methyl-D-aspartate receptors (NDMA) to increase calcium entry and transient receptor potential voltage receptor-1 (TRPV-1) receptors to increase sodium entry. The result is neuronal hyperexcitability. Cytokine molecules interact with glial cells, activating signaling pathways for the production of multiple other proinflammatory mediator molecules (PMM). These molecules will interact with neuronal and glial receptors to further drive inflammatory changes and increase neuronal hyperexcitability.

Ca^{2+} indicates calcium ions; CR, cytokine receptors; GLU, glutamate; Na^{+}, sodium ions; SP, substance P.

Proinflammatory cytokines (IL-1, IL-6, TNF-α)
Chemokines
Excitatory amino acids
Neuroactive peptides (substance P)
Saturated fatty acids
Prostaglandins
Nitric oxide
Reactive oxygen species (H_2O_2)
Nuclear transcription molecules (nuclear factor κB)

Table. Types of Proinflammatory Mediator Molecules (PMMs)

Not only do PMMs affect pain transmission, they also link peripheral nerve damage or inflammation to central nervous system inflammatory changes. They may be produced by either neurons or glial cells, and they exert receptor-mediated proinflammatory effects. IL indicates interleukin; TNF, tumor necrosis factor.

kinase C signaling pathways that displace the magnesium ion plug from *N*-methyl-D-aspartate (NMDA) receptors on postsynaptic neurons. The resulting intracellular calcium ion influx results in nitric oxide production, activation of proinflammatory genes, and decoupling of the G-protein from the MOR, all of which contribute to neuroimmune activation, hyperalgesia, and opioid tolerance[1,14] (Figure 3). Antidepressant drugs, such as amitriptyline,[15] and anticonvulsant drugs, such as pregabalin,[16] have some usefulness as adjunct therapy in the treatment of chronic pain by altering neurotransmitter release and neuronal cation uptake. Many patients with chronic pain do not benefit from this drug therapy, and therapeutic use is limited by side effects.

Frustration with the failure of available therapies and continued patient suffering has contributed to the search for a new theory to offer novel treatment approaches. The theory underlying neuroglial inflammation may provide new directions for treatment, predicated on empirical findings that explain the central inflammatory pathways reviewed in this section.

• *Neuronal Activation of Glia.* First, can peripheral neuronal activity from traumatic damage or inflammation ac-

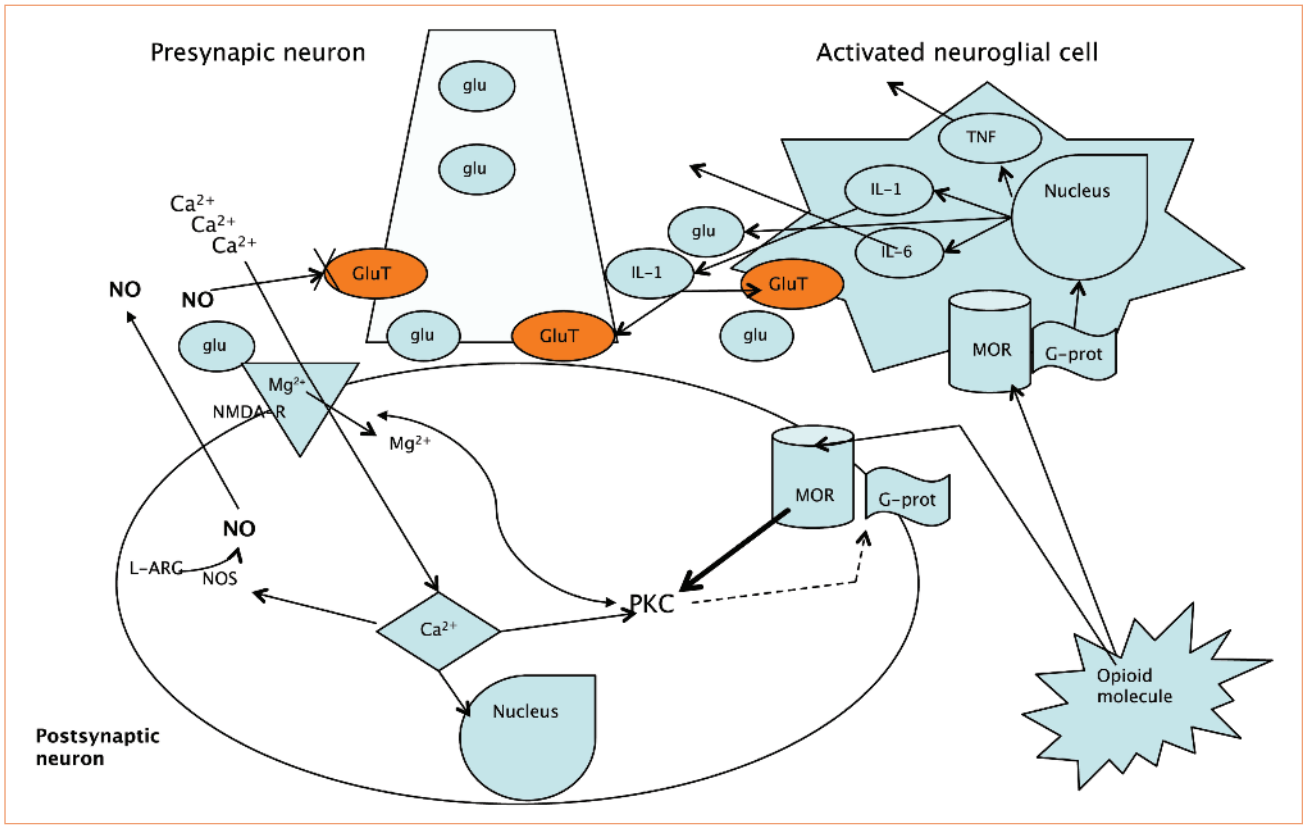

Figure 3. Opioid-Induced Neuroglial Activation

Long-term interactions of the opioid with its receptor results in activated protein kinase C (PKC) releasing the magnesium plug from the *N*-methyl-D-aspartate receptor (NMDA-R). The resulting calcium entry produces proinflammatory changes: nitric oxide (NO) is produced and diffuses to presynaptic neuron enhancing glutamate (glu) release; PKC is further activated and uncouples the G-protein (G-prot) from its mu opioid receptor (MOR); proinflammatory genes are activated. Glial cells are activated to produce proinflammatory cytokines by opioid-MOR interactions; these proinflammatory cytokines may further increase glu signaling by down-regulating the glu transporters (GluT).

Ca^{2+} indicates calcium ion; IL-1, interleukin-1; IL-6, interleukin-6; L-ARG, L-arginine; Mg^{2+}, magnesium ion; NOS, nitric oxide synthase; TNF, tumor necrosis factor-α.

(Adapted from DeLeo et al[1] with permission from Sage Publications.)

tivate the glial cells? Garrison et al[17,18] investigated this basic assertion in the first 2 experiments to examine the effect of peripheral nerve lesions on CNS function. In a rodent model of neuropathic pain, a chronic constriction lesion was produced in the sciatic nerve on 1 side by applying a ligature, and sham surgery was performed on the other side. Following onset of neuropathic pain behaviors, astrocytes on the affected side of the spinal cord, but not on the control side, were found to stain darkly using immunohistochemical methods, indicating inflammatory activation. When NMDA receptors on astrocytes were blocked with an antagonist before surgical intervention and ligature application, no astrocyte activation was observed. These observations are consistent with the proposed mechanism whereby astrocyte activation occurred when NMDA receptors responded to glutamate (an excitatory proinflammatory amino acid) molecules transported to the dorsal horn synapse and released from the damaged sciatic nerve axons in excessive amounts.[7] Since these early experiments, many studies using similar animal models have examined, verified, and replicated these and similar findings.[7,19-22]

It is generally believed that peripheral nerve damage as a consequence of trauma or inflammation results in an immune-mediated attack that generates PMMs.[23] Large nerves are composed of multiple discrete bundles of neural tissue separated by endoneural membranes. Resident in these large nerves are immune cells such as macrophages, Schwann cells, and fibroblasts kept separate from neural cytoplasm by the blood-nerve membranous barrier. Upon nerve injury associated with release of signaling molecules such as adenosine triphosphate and histamine, these innate cells are activated. They begin releasing multiple inflammatory molecules meant to target microbe invaders, but which also damage nerve structures and attract more immune cells. Innate cells are not programmed to recognize neural proteins P0 and P2 as "self"; thus, these molecules are attacked when

exposed by trauma, producing more damage. Some of the innate immune attack can be directed against myelin proteins and can evolve into a T-cell-mediated adaptive immune attack against the nerve structure.[24] As a consequence of this destructive self-directed immune activity, inflammatory molecules are generated that diffuse up the axon to the dorsal horn. Numerous activating molecules have been identified, such as intraneuronal cytokines and neuropeptides, and a chemoattractant known as fractalkine, bound to the presynaptic afferent neuronal membrane structure and released following intense afferent nociceptive activity. Release of these molecules into the dorsal horn of the spinal cord will activate the CNS immune cells via glial receptor interactions (Figure 2).

• *Proinflammatory Mediator Molecules and Glia.* The next proposed step in the theoretical model is that activated glial cells will produce proinflammatory cytokines and other PMMs. In response to receptor activation, genes in the nucleus of the glial cells that are responsible for production of proinflammatory molecules are turned on by PMMs from peripheral nerves and will continue the inflammatory cascade.[23] Microglia and astrocytes will begin producing proinflammatory cytokines and other PMMs (see Table). The literature has provided ample evidence to support this hypothesized sequence of events. In 2008, Henry et al[25] exposed mouse CNS microglial cells to lipopolysaccharide (an inflammatory component of bacterial cell wall, also known as LPS) in vitro. The microglial cells began producing cytokines—interleukins-1 and 6 (IL-1 and IL-6)—confirming that an immune stimulus could activate proinflammatory genes. Furthermore, brain microglial cells from animals given intraperitoneal LPS were found to produce messenger RNA (mRNA) specific for the proinflammatory cytokine molecules. Multiple preclinical studies have confirmed these findings over the last decade.[7,26,27] It is clear that glial cells can and do produce PMMs in response to inflammatory signals.

• *Neuroplasticity in Response to Proinflammatory Mediator Molecules.* To respond to PMMs with increased firing, neurons must be able to alter their activity, evincing neuroplasticity. Proinflammatory mediator molecules like the PICs, nerve growth factor, histamine, bradykinin, and prostanoids act directly via specific receptors on peripheral nociceptive terminals that innervate the tissues[28] (Figure 2). Acting as ligands at receptor sites specific to each molecule, postsynaptic neuronal signaling cascades have been identified that culminate in opening cation-permeable ion channels. For example, bradykinin molecules released from activated neuroglia act at B2 receptors on nociceptive neurons and, via a diacylglycerol-protein kinase C pathway, open sodium channels, enhancing afferent nociceptive nerve excitability and action potentials.[28]

The final model of neuroimmune activation is quite complex (Figure 2). Damaged or inflamed afferent neurons are releasing fractalkine and other PMMs into the synapse at the dorsal horn, causing receptormediated activation of glial cells. Similar to peripheral events, the proinflammatory cytokine molecules released from activated neuroglial cells interact with specific cytokine receptors on presynaptic and postsynaptic neurons in the dorsal horn, activating cation channels and increasing postsynaptic neuronal hyperexcitability.[9] Autocrine and paracrine cytokine interactions mediate production and release of more PMMs from neurons and glia, contributing to and driving a positive feedback loop of central inflammation that exacerbates and maintains chronic pain.[7]

• *Gene Therapies Targeting Neuroimmune Activation.* Further empirical evidence supporting the contributions of neuroimmune activation to pain is provided by a brief sampling of multiple recent investigations of gene therapies that target this process. Gene therapy involves the introduction of DNA or RNA into cells via viral or nonviral mechanisms.[29] The objectives of gene therapy are to suppress the production of deleterious proteins (such as proinflammatory cytokines) and to support the production of therapeutic proteins (such as interleukin-10 [IL-10], an anti-inflammatory cytokine). The latter approach is illustrated in a study by Sloane et al,[29] in which rats with neuropathic sciatic pain were injected intrathecally with naked plasmid DNA coding for IL-10. Following injection, an increase in intrathecal IL-10 gene expression, related to the time of administration and plasmid dose, coincided with observed decreases in pain behavior that lasted as long as 3 months. Milligan et al[30] conducted a similar experiment in which a replication-defective adenoviral expression vector was administered intrathecally to introduce IL-10-encoding DNA in the neuropathic rats. Again, pain behaviors decreased as IL-10 gene expression increased, but beneficial effects were more short-lived (1 to 2 weeks). Messinger et al[31] used antisense oligonucleotides (short nucleic acid polymers manufactured to bind to a complementary piece of DNA or RNA and prevent it from being used to produce proinflammatory proteins) to successfully treat thermal and mechanical hypersensitivity in a rat model of diabetic neuropathy. Using an epigenetic (changing gene expression without altering the underlying DNA sequence) approach, Chiechio et al[32] administered a histone deacetylase inhibitor in a mouse model of inflammatory pain. This upregulated transcriptional activity of nuclear factor-κB-regulated genes, which control metabotropic (receptors that mediate a cell metabolic function such as enzyme activation) glutamate-2 (MGLU2) receptor expression. The MGLU2 receptors decrease neurotransmitter release by primary afferent neurons in the spinal cord, and substantial analgesia was produced in animals receiving the histone inhibitor. It is clear that genetic therapies offer multiple potential approaches to the interruption of central neuroinflammation contributing to chronic pain.

Human Evidence Supporting the Theory

Although most studies supporting neuroimmune activation come from preclinical rodent models of neuropathic pain, evidence in populations with chronic pain syndromes has begun to emerge. Backonja et al[33] studied 14 adult subjects with posttraumatic neuralgia (type I and II complex regional pain syndrome [CRPS]) or other painful conditions in comparison to healthy controls. Plasma and cerebrospinal fluid (CSF) levels were analyzed for cytokine levels using enzyme-linked immunosorbent assay (ELISA). Two markers of inflammation—CSF interleukin-1β (IL-1β) and soluble tumor necrosis factor-α receptor (sTNFr)—were elevated in patients with CRPS, with a positive correlation to pain scores. Also, IL-10 was reduced in the CSF and blood of the pain group and was negatively correlated with pain intensity.

Alexander et al[34] studied 24 patients with CRPS compared with 16 "controls"—13 patients with painful conditions but not CRPS, and 3 patients with no pain. A statistically significant elevation in CSF levels of IL-6 and IL-1β was found in CRPS-affected patients but not in the control group. In 1 subgroup, there was a statistically significant correlation between CSF levels of IL-6 and pain visual analog scores. It is unclear why the "control" patients with pain not due to CRPS did not have altered inflammatory markers. Obviously there is much work to be done in elucidating these pathophysiological links, and increased understanding will follow further investigation.

Summary and Clinical Implications

The theory of neuroglial cell activation by peripheral nerve damage and its role in generating, exacerbating, and perpetuating chronic pain in a positive feedback cycle has been presented. Evidence supporting this theory comes primarily from preclinical studies originating in a wide selection of laboratories; several leaders in this field have presented much data supporting the theory. Human evidence is beginning to accumulate, but discrepancies in the data indicate the complexities underlying chronic pain syndromes.

The goal of interrupting the hypothesized positive feedback cycle of CNS neuroinflammation represents a clear direction for the clinical practitioners in pain management. However, this is a daunting task for human safety reasons. In animal models, the proinflammatory pathway is often identified by blocking 1 or more steps; for example, minocycline, which is known to block microglial cell metabolism, may be administered before the production of a peripheral nerve lesion, and neuropathic pain behaviors and inflammatory markers will be prevented.[9] Following established neuropathic pain from a sciatic lesion, the administration of the compound fluorocitrate or propentofylline, known to block astrocyte metabolism, can reverse the pain behaviors.[9] But are these compounds safe for use in humans? Fluorocitrate blocks aconitase, a metabolic enzyme used in the tricarboxylic acid cycle in astrocytes. Would blocking these neuroimmune cells, essential to the regulation of synaptic milieu, be irreversible and produce deleterious consequences for the human host? Furthermore, these neuroimmune cells may have constitutive—that is, essential regulatory—functions that are currently unknown. The challenges facing pain research in this area are enormous and fraught with potential dangers for human subjects.

The future of pain medicine may well lie in the identification and treatment of inflammatory pathways that drive debilitating chronic pain syndromes. Multiple target molecules exist (see Table), and intrathecal and epidural delivery is relatively easily accomplished. Although a dearth of clinical studies exists, preclinical investigations are yielding new directions. Of note, emerging evidence may indicate a novel role for human recombinant erythropoietin (rhEPO) in anti-inflammatory, antinocieptive pathways.[35-37] The previously identified neuroprotective role of this molecule in spinal cord injury models may expand to include antinociception if rhEPO can consistently block central inflammatory pathways by down-regulating nuclear factor-κB (see Table), demonstrated in a recent preclinical investigation.[35] Clearly there is an urgent need for phase 1 trials of CNS-targeted anti-inflammatory therapies. Finally, genetic therapies targeting inflammatory pathways are providing pain researchers with a new, exciting, and promising direction for treatment of chronic pain. Hopefully, attempts to control peripheral and central inflammation will soon bring hope to the millions of patients faced with severely decreased quality of life due to chronic pain and its consequences.

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