REVIEW ARTICLE

The Role of Glia and the Immune System in the Development and Maintenance of Neuropathic Pain

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■ **Abstract:** Neuropathic pain refers to a variety of chronic pain conditions with differing underlying pathophysiologic mechanisms and origins. Recent studies indicate a communication between the immune system and the nervous system. A common underlying mechanism of neuropathic pain is the presence of inflammation at the site of the damaged or affected nerve(s). This inflammatory response initiates a cascade of events resulting in the concentration and activation of innate immune cells at the site of tissue injury. The release of immunoactive substances such as cytokines, neurotrophic factors, and chemokines initiate local actions and can result in a more generalized immune response. The resultant neuroinflammatory environment can cause activation of glial cells located in the spinal cord and the brain, which appear to play a prominent role in nociception. Glial cells, also known as neuroglia, are nonconducting cells that modulate neurotransmission at the synaptic level. Glial cells can be subdivided into two primary categories: microglia and macroglia, which include astrocytes and oligodendrocytes. Astrocytes and microglia are known to play a role in the development, spread, and potentiation of neuropathic pain. Following peripheral nociceptive activation via nerve injury, microglia become activated and release pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin-1β, and interleukin-6, thereby initiating the pain process. Microglia propagate the neuroinflammation by recruiting other microglia and eventually activating nearby astrocytes, which prolongs the inflammatory state and leads to a chronic neuropathic pain condition. Our review focuses on the role of glia and the immune system in the development and maintenance of neuropathic pain. ■

Key Words: neuropathic pain, hyperalgesia, neurotrophic factor, neurotransmitters, gene expression, sensory receptors, astrocytes, microglia, nociceptors

INTRODUCTION

Neuropathic pain refers to a variety of chronic pain conditions with different underlying pathophysiologic mechanisms. Neuropathic pain can originate from neuronal tissue damage or a dysfunction in the nervous system.[1,2] The abnormal perception of neuropathic pain is characterized as being allodynic (a typically nonpainful stimulus is perceived as painful), hyperalgesic (a normally painful stimulus is exaggerated) or as spontaneous (shock-like, stabbing or burning pain sensations that are unrelated to a known stimulus).[3] The sensation of the neuropathic pain may or may not be localized to the dermatomal distribution of the affected nerve(s).

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Recent studies indicate that a communication exists between the immune system and the nervous system[4]. Although multiple conditions may generate neuropathic pain, a common underlying mechanism is the presence of inflammation at the site of the damaged or affected nerve(s). This inflammatory response initiates a cascade of events resulting in increased local perfusion, increased capillary permeability, and concentration and activation of innate immune cells at the site of tissue injury, irritation, or infection. Immunoactive substances, such as cytokines, neurotrophic factors, and chemokines, released at the site of injury have local actions and can initiate a systemic immune response. The resultant neuroinflammatory environment can cause activation of microglia and astrocytes, glial cells located in the spinal cord and brain, which appear to play a prominent role in nociception.

THE SUPPORTING CELLS OF THE NERVOUS SYSTEM: MICROGLIA AND ASTROCYTES

Neurons and glial cells are two cell types in the nervous system that have close interactions on a cellular and molecular level. Neurons are cells specialized to conduct electrochemical impulses. Glial cells, also known as neuroglia, are nonconducting cells that were initially only known to provide support; however, recent evidence has shown that glial cells also provide nutrition, protection, and insulation to the neurons of the central nervous system (CNS). Some glial cells are also known to modulate neurotransmission at the synaptic level.[5] Glial cells constitute 70% of the total cell population in the brain and the spinal cord.[6] Glial cells can be subdivided into two primary categories: microglia, comprising 5% to 10% of the glial population, and macroglia, which include astrocytes and oligodendrocytes.[7] Furthermore, astrocytes and microglia are known to play a role in the development, spread, and potentiation of neuropathic pain.[8–16]

When myeloid progenitor cells migrate to the peripheral nervous system (PNS), they may differentiate into dendritic cells or macrophages. However, when the same bone marrow-derived progenitor cells travel to the CNS, they differentiate into microglia which act similarly to macrophages when they are activated.[17–19] Under normal homeostatic conditions, microglia are in a resting, sessile state and have small soma with fine or thin-branched processes. Microglial cells migrate to the central terminals of afferent peripheral nerves responding to pain signals and undergo activation. Upon activation microglia undergo a number of morphological and functional changes facilitating isolation of injured cells and eliminating potential pathogens[20] (Figure 1). These changes include mobilization and proliferation and induce phagocytic ability of microglia.[21] At the site of injury, activated microglia can project processes, through an adenosine 5′-triphosphate (ATP)-mediated elongation, in order to isolate the injured cells.[20] Although microglia have a homogeneous distribution in the CNS, only microglia in the spinal cord are activated following peripheral nerve injury.[22]

After peripheral nerve injury, proliferation of activated microglia was found on the ipsilateral dorsal horn (DH), while the contralateral DH and naive animals displayed weak microglial activation.[23,24] Activated microglia also display a change in surface markers, membrane bound or embedded proteins, compared with

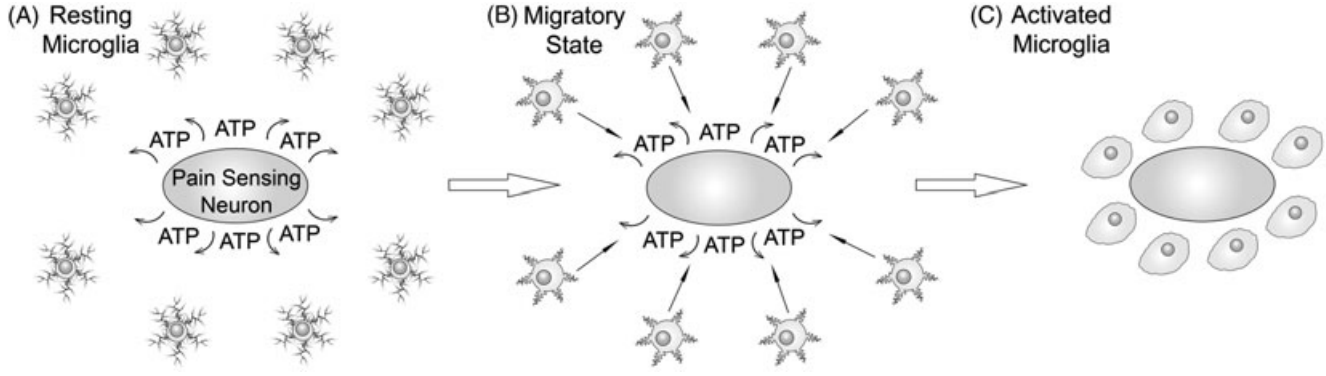

Figure 1. (A) Following a peripheral injury, the synaptic projection of a pain sensing neuron within the spinal cord releases ATP. (B) Nearby microglial cells within 50 to 100 μm are drawn to the source of ATP and undergo morphological changes as they approach the source and become activated. (C) Fully activated microglial cells are localized around the pain sensing neuron and begin to interact with the neuron on a molecular level, releasing various neuroinflammatory agents. ATP, adenosine 5′-triphosphate.

resting stage microglia. Activated surface markers can include complement receptor 3 (also known as CD11b/CD18, Mac-1, ITGAM or integrin alpha-M),[25–28] which is involved in phagocytosis,[29] toll-like receptor 4, which is involved in pathogen recognition,[16] CD14, also involved in pathogen recognition,[16] CD44 involved with adhesion and migration,[30] and up-regulation of MHC I and II,[30] which are involved in antigen presentation to T cells.[10] These better prepare the glial cells to eliminate invading microbes and to aid in phagocytosis. Of note, the function and morphological changes are not always temporally related, as it may be possible for migration or phagocytosis to occur before or after contraction of the processes.[29] The activation of microglia additionally triggers the secretion of a variety of signaling peptides such as cytokines, neurotrophic factors, and chemokines. The production and subsequent release of pro-inflammatory cytokines like interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNFα) from activated microglia cells lead to the activation of neighboring astrocytes.[31]

Astrocytes make up the majority of glial cells in the CNS; however, the development and function of astrocytes remains largely uncharacterized.[32] Astrocytes are phagocytic cells that play an important role in neuronal development as well as in establishing and maintaining the blood brain barrier (BBB).[32] In a resting state, astrocytes isolate neurons and oligodendrocytes to help maintain the microenvironment of the CNS by regulating extracellular ion concentrations of K^+ and Ca^{2+} as well as neurotransmitter concentrations via uptake. In a basal state, astrocytes have thin processes. Upon activation, these cells undergo hypertrophy, proliferate, and increase expression of intermediate filaments such as the glial fibrillary acidic protein, an astrocyte-specific activation marker.[29,33] These functions provide important links to antigen presentation to T cells and may aid T cell crossing the BBB.

The activation of astrocytes results in the prolongation of a pain state. Resting astrocytes express basal levels of cytokine receptors. IL-1β and possibly interleukin-18 (IL-18), released from activated microglial cells, bind to interleukin-1 (IL-1) receptors located on the astrocyte membrane, inducing a series of intracellular events culminating in the activation of the astrocyte.[34,35] TLR, expressed in microglial cells, may trigger the synthesis of IL-18, a member of the IL-1 family, via the activation of p38 mitogen activated protein kinase (p38MAPK), known to induce expression of pro-inflammatory cytokines such as IL-1β and IL-6. Miyoshi et al. reported that intrathecal injection of IL-18 induces tactile allodynia and astrocyte activation.[34] These intracellular events result in the secretion of IL-1β, IL-6, and TNF-α by astrocytes, as well as the expression of inducible nitric oxide (NO) synthetase, to further propagate the inflammatory response and prolong the pain state.

Another unique characteristic of astrocytes is their role in both the deactivation of glutaminergic activity by the uptake of extracellular glutamate and the synthesis of glutamate from glucose. Glutamate (the main excitatory neurotransmitter in the brain and the spinal cord) content increases in the DH during chronic pain. Pyruvate carboxylase, an enzyme involved in the synthesis of glutamate, is expressed in astrocytes but not in neuronal cells.[36] Glutamate activates several ionotropic and metabotropic membrane receptors. Of particular interest is the ionotropic N-methyl-D-aspartate receptor because of its crucial role in central sensitization of spinal cord nociceptive neurons as well as activation of astrocytes via an influx of Ca^{2+} into the cells.[37] An influx in Ca^{2+} has an important role in signaling pain by promoting neurotransmitter release and modulating cell membrane excitability.[38]

HOW GLIAL CELLS BECOME ACTIVATED AFTER PERIPHERAL NERVE INJURY

The activation of microglia and astrocytes can occur following physiological changes in the body, such as trauma in the CNS, ischemia, inflammation, and infection. The activation of these glial cells is most often implicated in the development, spread, and potentiation of neuropathic pain.[8–16] Microglia and astrocytes are generally activated in the DH after a peripheral nerve injury occurs. After receiving a pain stimulus, peripheral neurons transmit "pain" signals to the DH of the spinal cord, releasing neurotransmitters such as calcitonin gene-related protein (CGRP), substance P, glutamate, gamma amino butyric acid, serotonin (released from descending pain pathways), and ATP. These neurotransmitters initiate the activation of glial cells in the area of the synapse, further sensitizing postsynaptic neurons (Figure 2). Various mechanisms by which glial cells are activated have been suggested. These include

1. Chemical mediators including substance P, CGRP, NO, purinergic agents (such as ATP), glutamate, and endogenous opioid peptides released at the time of injury travel through or between afferent neurons, not only affecting synaptic transmission but also activating glial

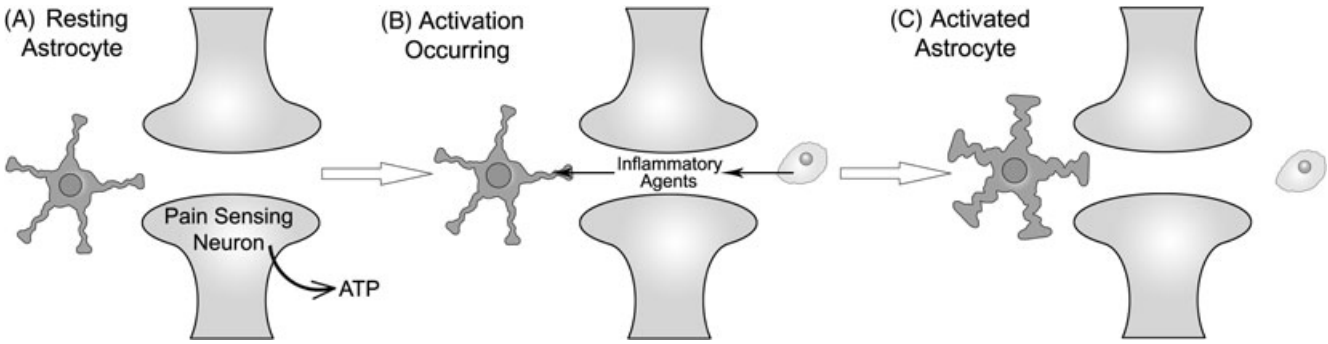

Figure 2. (A) A pain sensing neuron releases ATP which draws in microglial cells and induces microglial activation. (B) Activated microglial cells begin to release various inflammatory agents that sensitize neurons as well as activate astrocytes. (C) Astrocytes become activated in response to microglial release of inflammatory agents, undergo hypertrophy, and begin to potentiate the chronic pain state. ATP, adenosine 5′-triphosphate.

cells.[39–44] ATP released by afferent neurons causes migration and activation of microglia within a range of 50 to 100 μm, producing an intracellular increase in Ca^{2+} and brain-derived neurotrophic factor (BDNF), which results in the activation and translocation of NF-κB to the nucleus-initiating expression for numerous pro-inflammatory agents. NO acts similarly upon NF-κB in astrocytes affecting gene expression and inducing activation.[39,40,45,46] Sensory neurons undergoing painful stimuli causes release of substance P (or via NO-stimulated production of substance P) which activate glial cells in the CNS by activation of neurokinin-1 (NK-1) receptors. Other chemical mediators, like purinergic agents, glutamate, and opioid peptides, induce activation of glial cells by direct interaction with specific membrane receptors (Figure 3).

2. Glial activation can occur via shifts in intracellular and extracellular ion concentrations. An increase in afferent neuronal input causing an elevation of extracellular K^+ leads to increased K^+ uptake by astrocytes, resulting in membrane depolarization, morphologic changes, and possibly activation.[47] Furthermore, K^+ has been shown to induce microglial activation in rat hippocampal tissue *in vitro*.[48] Similarly, an influx of Ca^{2+} results in the activation of both astrocytes and microglia, with concomitant changes in morphology and cellular function. Pro-inflammatory agents generated and released by activated glial cells can further activate nearby glial cells (Figure 4).
3. Previous studies have shown that peripheral injury results in astrocyte activation in the trigeminal complex of the brain stem.[49,50] Interestingly, proximal blockade of primary afferent input following a peripheral nerve injury fails to inhibit glial activation at both the spinal and supraspinal levels. These studies suggest that supraspinal-activated astrocytic cells may potentially modulate neuropathic pain by further activation of glial cells in the spinal cord via descending pathways.[51]
4. It has been shown that increased permeability of the BBB after an injury allows peripheral macrophages to migrate, proliferate, and differentiate into activated glial cells in the brain.[50–53] In addition, peripherally generated inflammatory agents, outside of the neuronal afferent pathway, can activate glial cells in the CNS. For example, a proximal anesthetic block fails to inhibit either spinal cyclooxygenase gene expression or prostaglandin E2 release into the cerebrospinal fluid (CSF).

Interestingly, acute pain, such as a paper cut or a needle prick, will not activate glial cells.[40] However, following a more serious injury, glial cells exhibit dynamic plasticity and switch from a resting state to become active in the modulation of neuronal activity.[54] Once activated, glial cells change their morphology, via hypertrophy and potentially retraction of the processes, and synthesize specific cell markers and kinases, some having an active role in initiating and potentiating an immune response.

ROLE OF GLIAL ACTIVATION

Both microglia and astrocytes are involved in neuropathic pain pathways. After a threshold stimulus, acti-

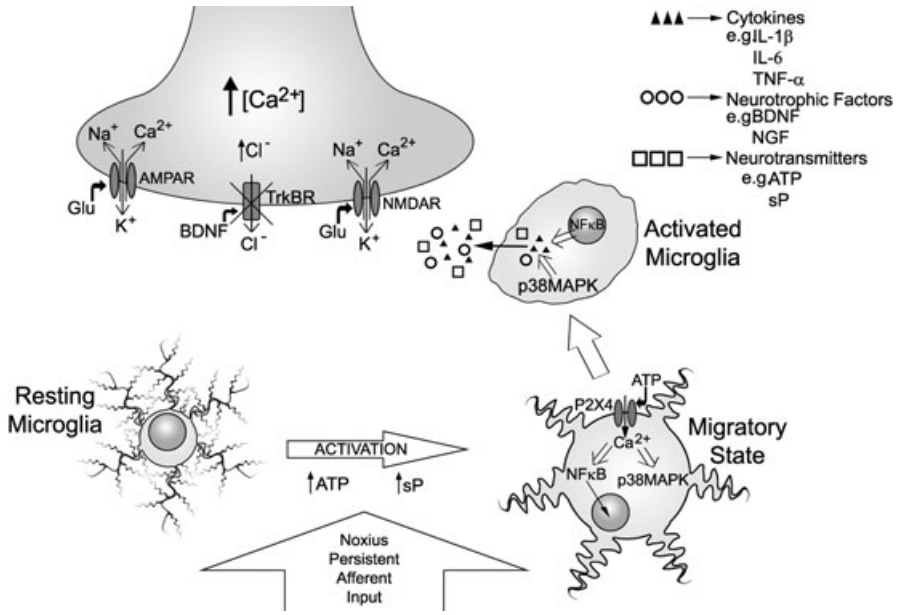

Figure 3. Noxious afferent input results in the activation of resting microglial cells that migrate to the source of ATP. ATP can bind to the P2X4 receptors on the microglial surface, which results in an increase in intracellular Ca^{2+} within the cell. The influx of Ca^{2+} results in the translocation of NFκB to the nucleus and induction of the p38MAPK pathway. The nuclear form of NFκB and induction of the p38MAPK pathway initiates transcription of various neuroinflammatory agents including cytokines, neurotrophic factors, and neurotransmitters. The release of these neuroinflammatory agents into the syntaptic cleft and subsequent binding to various receptors result in an increase in intracellular ions within the neuron, such as Ca^{2+} and Cl^{-}, which depolarizes the cell and thereby causing sensitization. Two prominent receptors that are involved with Ca^{2+} influx into the neuron are AMPA and NMDA receptors. BDNF has been shown to bind to the TrkB receptor and inhibits the efflux of Cl^{-} out of the neuron. ATP, adenosine 5′-triphosphate; BDNF, brain-derived neurotrophic factor; p38MAPK, p38 mitogen activated protein kinase.

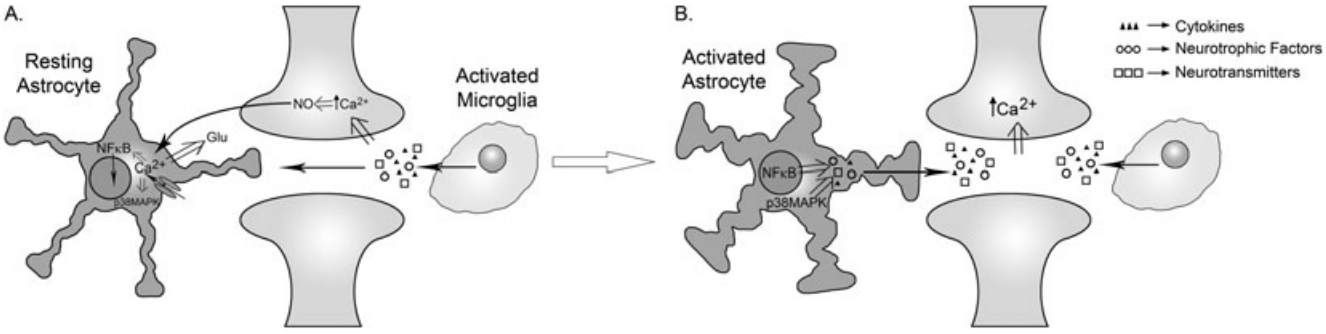

Figure 4. (A) When activated microglial cells release neuroinflammatory agents into the synaptic cleft, local astrocytic surface receptors bind to various agents and result in an influx of Ca^{2+} Microglial cells can cause expression of nitric oxide synthase within the postsynaptic neuron, which freely diffuses through cell membranes and can also induce astrocyte activation and result in Ca^{2+} influx. High levels of intracellular Ca^{2+} result in translocation of NFκB from the cytoplasm to the nucleus of astrocytes, induction of the p38MAPK pathway, as well as a dose-dependent release of glutamate. (B) Upon activation, the astrocyte undergoes hypertrophy and increased production of neuroinflammatory agents that are secreted into the synaptic cleft. Astrocyte activation in conjunction with microglial activation significantly depolarizes the neuron increasing its sensitivity and potentiating the neuropathic pain state. NO, nitric oxide.

vated glial cells release inflammatory stimulants such as cytokines, prostaglandins, neurotrophic factors, ATP, NO, D-serine, and glutamate.[10,44,50,55–59] These inflammatory stimulants play a critical role in the development and maintenance of central sensitization and hyperalgesia[40] by altering the polarization characteristics of the afferent neurons and thus modulate the transmission of painful stimuli to the CNS.[60,61] For example, astrocyte activation leads to increasing intracellular Ca^{2+} which stimulate a calcium-dependent glutamate release,

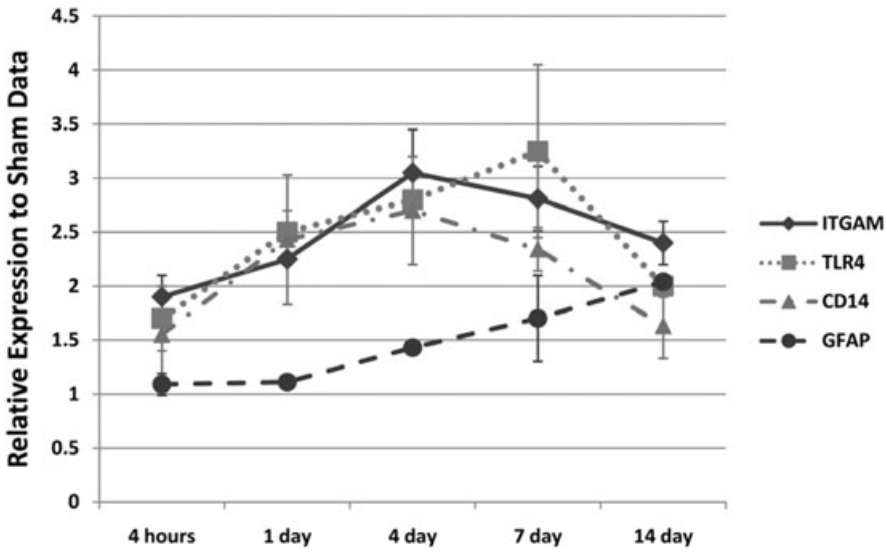

Figure 5. Expressions of glial activation markers following L5 spinal nerve transection were normalized to sham animals. Markers of microglial activation (ITGAM, TLR4, and CD14) increased significantly relative to sham animals within the first 4 hours after nerve transection, which peaked at 4 to 7 days and began to decline. Astrocyte activation (GFAP) lagged 1 day behind microglial activation and continued through day 14. These trends in glial activation markers continued through day 28, although sham data were not collected and thus not graphed. This corroborates the concept that microglia are important initiators of neuropathic pain development, whereas astrocytes contribute to the prolongation of a pain syndrome. Data extracted from Tanga et al.[68]

resulting in an inward current produced in adjacent neurons (Figure 4).[62]

Current research suggests that microglia are involved in the early development, whereas astrocytes function to sustain neuropathic pain.[16,63–65] Microglial activation leads to the release of signaling proteins, such as IL-1β, into the cell interstitium and to some extent the CSF. These signaling proteins bind to specific sites on the astrocyte membrane initiating cell activation.[66,67] Upon activation, a positive feedback cycle occurs whereby astrocytes release inflammatory mediators, e.g., TNF-α, which in turn can activate other glial cells.[66] Astrocyte activation is accompanied by a decrease in microglial activity over time (Figure 5).[68] In an animal model, intrathecal administration of activated microglial cells decreased pain threshold while a similar application of activated astrocytes did not,[69] further demonstrating that activated astrocytes are not predominantly involved in the development of a pathological pain state, but rather potentiation of a pain state.

CYTOKINES AND THE INFLAMMATORY RESPONSE

The process of inflammation resulting from an infection or injury is a physiological response that acts as part of the body's defense mechanism. Inflammation is traditionally associated with various symptoms: redness, swelling, heat, and pain. On the cellular level, inflammation involves the body's innate immune system, including activated microglia and astrocytes, macrophages of the CNS.[70] Upon activation, these macrophages and other innate immune cells release immunoactive agents, which mediate the inflammatory response, including pro- and anti-inflammatory cytokines.[71] As their name suggests, pro-inflammatory cytokines maintain an up-regulated inflamed response. In animal models, pro-inflammatory agents such as TNF-α, carrageenan, or complete Freund's adjuvant injected around the sciatic nerve (directly or indirectly) induced mechanical allodynia,[72] supporting the crucial role of inflammation in the development and maintenance of neuropathic pain. TNF-α, IL-1β, and IL-6 have all been identified as pro-inflammatory cytokines.[73] Conversely, anti-inflammatory cytokines, such as IL-10, down-regulate the inflammatory process. In fact, research indicates that IL-10 is a very potent anti-inflammatory cytokine as demonstrated in virtually all animal models of chronic pain.[31]

Cytokines are very potent small proteins produced by either immune (macrophages or helper T cells) or non-immune cells (endothelial cells or Schwann cells) that function as cellular communicators at an autocrine or paracrine level. Most cytokines are pleiotropic in

nature, and different cytokines may have similar functions. Under inflammatory conditions, cytokines are produced and released to act on other cells, often as part of a cytokine cascade (Figure 2).[74] Under normal conditions, the production of both pro- and anti-inflammatory cytokines aids the immune system in destroying a pathogen and healing the damaged tissue. However, prolonged pro-inflammatory cytokine release may lead to a pathological response such as chronic pain.[74] Congruent to this line of evidence, cytokine antagonists have been used to demonstrate the involvement of these cytokines in the initiation of a pathological pain state.[8,12,75]

Following a peripheral nerve injury, Schwann cells and macrophages secrete cytokines to initiate the healing process of the injured nerve. The initial inflammatory response requires increased localized production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and decreased production of anti-inflammatory cytokines such as IL-10.[74] Not only are pro-inflammatory cytokines elevated in a pain state, but all three aforementioned pro-inflammatory cytokines have the ability to increase neural activity and mechanosensitivity at the dorsal root ganglia (DRG).[76] Mechanical allodynia and hyperalgesia in a rat pain model have been associated with early increased levels of TNF-α, IL-1β, and IL-6 and delayed expression levels of IL-10.[77]

Cytokines may also be transported to the DRG and must cross the BBB to reach the DH of the spinal cord, causing activation of microglia and astrocytes. Proposed mechanisms by which cytokines cross the BBB is by active transport,[78–80] by passage through weak areas of the BBB,[81] and by binding to blood vessel receptors that run through the brain, thus stimulating release of secondary messengers such as prostaglandins inside the CNS.[82] For example, elevated levels of IL-1β and IL-6 have also been found in the CSF in patients with complex regional pain syndrome.[83] Upon interaction with specific receptors, inflammatory cytokines initiate a cascade of intracellular events that result in the production of prostaglandins or sympathomimetic amines, which may be the substances ultimately responsible for the enhanced nociception.[84–88]

Specific functions for some of the previously mentioned pro-inflammatory cytokines have been identified. TNFα is one of the most prominent pro-inflammatory cytokines involved in the initiation of the inflammatory response and chronic pain including thermal hyperalgesia.[89,90] There are two TNFα receptors termed p55 and p75. TNFα binding to the p55 receptor induces programmed cell death while binding to the p75 receptor results in translocation of nuclear factor kappa B to the nucleus where it binds to promoter regions in several genes. TNFα binding stimulates production of IL-1β, which in turn induces expression of IL-6 and other pro-inflammatory cytokines.

Another cytokine that is known to have a crucial role in the development of chronic pain is IL-1β. IL-1β receptors (IL-1R) are located in the CNS and the PNS. The interaction between IL-1β and its receptor induces a series of intracellular biochemical events that involve expression of IL-6 (another pro-inflammatory cytokine), p38MAPK, and NFκB, which are ultimately responsible for inducing gene expression of cyclooxygenase-2 (COX-2) and type II phospholipase A2. The main effect of IL-1β is the transcription of COX-2 with little effect on COX-1. After COX-2 induction, prostaglandin E2 is produced in large amounts and mediates many of the biological activities of IL-1β. In addition, induction of phospholipase A2 releases arachidonic acid, which is the rate limiting step in the synthesis of prostaglandins and leukotrienes.[91] Production of prostaglandin E2 and I2 induces sensitization of nociceptors.[82] Studies showed prostaglandin expression, induced by intraplantar injection of IL-1β, caused a dose-dependent bilateral thermal and mechanical hyperalgesia,[92–94] which was inhibited by pre-treatment with nonsteroidal anti-inflammatory drugs.

Typically, IL-6 is thought to be a pro-inflammatory cytokine capable of causing hyperalgesia by induction of arachidonic acid release. Interestingly, IL-6 has been shown to suppress *in vitro* production of IL-1β by monocytes/macrophages stimulated with lipopolysaccharide (LPS) while *in vivo* it stimulates the production of anti-inflammatory proteins IL-1β receptor antagonist and soluble TNFα receptor; two molecules that act as decoys preventing the binding of IL-1β or TNFα with the cell receptors.[95–99] IL-6 is discussed in more detail under neuropoietic cytokines.

CHEMOKINES AND NEUROPATHIC PAIN

A large subfamily of cytokines are chemotactic cytokines, generally referred to as chemokines. Characteristics common among all chemokines include both structural and functional features: the conservation of a cysteine motif in the N-terminal region of the protein and the induction of their effects via various 7-transmembrane G-protein-coupled receptors. Chemokines play a dual role in the immune system; they

act as chemoattractants during inflammation and as traffickers of hematopoietic stem cells during development and differentiation.[100,101] Chemokine binding to receptors triggers downstream signaling cascades that ultimately result in Ca^{2+} influx.[102–104]

Chemokines can be synthesized throughout the body from a wide variety of cells with expression being variable depending on the immunological state. Some chemokines are homeostatic, such as those that guide lymphocytes to lymphoid tissues, while others are only expressed to facilitate the localization of the immune response around the site of injury or infection.[105] Numerous rodent neuropathic pain models have demonstrated an up-regulation of the chemokine receptors CX3CR1 or CCR2 as well as monocyte chemoattractant protein-1 (MCP-1; recently termed CCL2) in neural tissues after an injury occurs such as the partial ligation of the sciatic nerve,[106–108] chronic constriction injury of the sciatic nerve,[40,44,109] chronic compression of the L4-L5 DRG,[110,111] bone cancer pain,[112] and zymosan-induced inflammatory pain.[40,113,114] The importance of the chemokine receptor CCR2 in neuropathic pain may be very significant as evidenced in genetically engineered mice lacking the CCR2 gene. These mice failed to display a detectable change in acute pain behavior for mechanical hyperalgesia after partial ligation of the sciatic nerve.[106]

Chemokines' ability to alter nociception can occur via induction by pro-inflammatory agents. For example, chemokines were expressed by endothelial cells after administration of either a lipopolysaccharide, IL-1β, or TNF-α.[115] Furthermore, chemokines and their receptors have been shown to facilitate pain via injection of stromal cell-derived factor-1α (SDF1α/CXCL12), Regulation upon Activation, Normal T cell Expressed, and Secreted (RANTES/CCL5), or macrophage inflammatory protein-1α (MIP1α/CCL3) into the noninflamed rat hindpaw inducing dose-dependent tactile allodynia.[103] Chemokines such as SDF1/CXCL12 acting on neurons and/or astrocytes are believed to affect the release of glutamate, potentially affecting neuronal excitation.[116,117] Application of RANTES/CCL5 or MCP-1/CCL2 to DRG cultures have been shown to result in the release of substance P[103] and CGRP,[118] respectively. Both substance P and CGRP are potent peptides and neurotransmitters with established roles in pain transmission. MCP-1/CCL2 produces membrane threshold depolarization and action potentials in neuronal cell bodies.[110,111,119] These excitatory effects on sensory neurons are believed to facilitate the release of CGRP.[118] Furthermore, the increase in electrical activity after a peripheral injury occurs may stimulate the release of MCP-1/CCL2 into the DH of the spinal cord, further activating CCR2 bearing glial cells and/or neurons.[44,106,120]

NEUROTROPHIC FACTORS AND NEUROPATHIC PAIN

Neurotrophic factors are protein molecules that promote the survival, growth, and maintenance of neurons. Upon tissue injury neurotrophic factors act to prevent damaged neurons from initiating programmed cell death. The term neurotrophic factor describes three major families including neurotrophins, glial cell line-derived neurotrophic factor (GDNF) family, and neuropoietic cytokines.

In mammals there are only 4 members of the neurotrophin family consisting of nerve growth factor (NGF), BDNF, neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4).[121] Under normal physiological conditions, neurotrophins are secreted by peripheral targets (such as skin, muscle, and viscera) and transported retrogradely to the neuron cell body.[122,123] Both BDNF and NT-3 also undergo anterograde transport to neurons and target cells, thereby potentially acting as a neuromodulator and trophic factor.[124] Furthermore, activated astrocytes within the brain have been shown to be a source of NGF, BDNF, and NT-3 around the site of an injury.[125] NT-4 has been shown to be synthesized by most neurons of the DRG and dorsal and ventral horns.[126]

Of the neurotrophins, NGF has been the most extensively studied. Aside from its role in developing nervous tissues, NGF is most commonly known for its role as a major regulator of inflammatory and homeostatic pain states.[89,127–132] Elevated NGF levels enhance expression of the neuropeptides substance P and CGRP.[129,133–138] Thermal hyperalgesia and mechanical allodynia, in both animal and human studies, have been linked to elevated NGF.[89,90,139,140]

Expression of NGF is often seen as biphasic with the secondary increase seen to correlate temporally with IL-1β expression.[141] Further evidence supporting the secondary increase correlating to IL-1β expression, likely released by macrophages, was characterized in three ways. First, by a temporal relationship between macrophage invasion and the secondary increase in NGF. Second, it was mimicked *in vitro* using activated macrophages or recombinant IL-1β. Finally, inhibition

of the secondary expression of NGF was seen when using IL-1β antibodies.[141,142]

Unlike NGF, BDNF is expressed at basal levels in sensory neurons. BDNF, once neuronally synthesized, is transported anterogradely to the spinal cord in secretory vesicles[143–145] in addition to the target-derived BDNF that is retrogradely transported to the cell body.[145] Under inflammatory conditions, BDNF is regulated in an NGF-dependent fashion[146–148] and appears as an important mediator of centrally sensitized inflammatory pain via inhibition of chloride ion excretion resulting in further depolarization of the neuron.[144,149–151] TNF-α up-regulates expression of BDNF in primary astrocytes, those astrocytes activated earliest, during inflammation.[152] Studies seem to indicate that BDNF signals through the DRG to the dorsal horn of the spinal cord.[150,153] A direct role for BDNF in the generation of neuropathic pain was demonstrated by administration of exogenous BDNF which subsequently induced both thermal hyperalgesia and mechanical allodynia.[154–156]

In neuropathic pain, NT-3 has an anti-inflammatory role by modulating injury-associated increases in BDNF,[157] substance P,[158,159] and IL-6.[160] NT-3 also antagonizes the NGF pro-inflammatory pathway, inhibiting the production of pro-inflammatory mediators such as NO, TNF-α, and IL-1β.[161–164] Furthermore, studies have shown that nerve injury-induced phenotypic changes in the DRG neurons can be reversed by exogenous NT-3 and is evidenced by decreased expression of BDNF.[157]

Currently, little is known about the specific role of NT-4 in regards to glial response and the immune system. One study showed that like BDNF, NT-4 can sensitize sensory afferent neurons to thermal stimulation.[165] However, NT-4 antibodies failed to abolish thermal hyperalgesia in a chronic pain model,[166] indicating that NT-4 plays a supporting role. The specific role of NT-4 in inflammatory pain has yet to be fully elucidated.

The GDNF family of neurotrophic factors is composed of secreted proteins that are structurally related; these are GDNF, neurturin, artemin, and persephin. In regards to neuropathic pain, the GDNF family has not been as intensely studied as the neurotrophins. Most research for the GDNF family has centered on GDNF while artemin, neurturin, and persephin are still considered a fairly new area of research. GDNF is expressed in almost all tissue types such as spinal cord, cartilage, stomach, intestine, and kidneys and includes every region of the brain.[167] Artemin, neurturin, and persephin are expressed in many tissues throughout the body, although typically at very low levels.[134,168]

GDNF's primary role seems to be in the repair or neuroprotection of nerves after an injury occurs. In one study, GDNF was released by activated microglia causing a restoration of locomotor function after LPS-induced inflammation.[169] A separate study showed that administration of IL-1β, interferon-γ, TNF-α, or LPS on cultured astrocytes increases GDNF expression, indicating that GDNF in astrocytes is regulated by inflammatory stimuli.[125] Partial sciatic nerve ligation and spinal nerve ligation, both established pain models, have been shown to induce inflammation, resulting in mechanical allodynia and thermal hyperalgesia. After either injury, intrathecal GDNF treatment showed a significant increase in withdrawal thresholds for mechanical and thermal stimulation showing GDNF's ability to attenuate hyperalgesia.[170] Similarly, artemin injections produced a time and dose-related reversal of tactile and thermal hypersensitivity, which was maintained with sustained artemin administration.[171] However, conflicting data have since shown that intrathecal artemin injections fail to inhibit the development of hyperalgesia after nerve ligation.[172]

Interestingly, studies show that expression of GDNF, artemin, and neurturin was enhanced after neuronal injury[173–175] or chemically induced inflammation.[169,176,177] There is significant debate on the involvement of GDNF, artemin, and neurturin in thermal hyperalgesia revolving around transient receptor potential vanilloid 1 (TRPV1) expression.[176–178] Both NGF, a pro-inflammatory neurotrophic factor, and GDNF, an anti-inflammatory neurotrophic factor, have been shown to increase TRPV1 expression, a protein known to facilitate thermal hyperalgesia.[176] However, studies suggest that NGF and GDNF act on distinctive neuronal cell populations to induce TRPV1 expression.[176]

Very little is known regarding persephin and its role in neuropathic pain. The entire GDNF family, with the sole exception of persephin, showed increased gene expression following administration of capsaicin, a known activator of TRPV1.[179] More research is needed to determine any role that persephin plays in neuropathic pain.

The neuropoeitic cytokine family consists of IL-6, IL-11, leukeaemia inhibitory factor (LIF), oncostatin-M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), and neuropoietin. The neuropoietic cytokine

family has two general unifying characteristics. First, neuropoietic cytokines all have a degree of homology to IL-6, and second, they share a common signal-transducing receptor, glycoprotein 130 (gp130). Given that all neuropoietic cytokines rely on gp130 to induce their cellular response, it is important to note that gp130 has been shown to be up-regulated in peripheral nerves, DRG, and spinal cord in a variety of pain models.[180] Neuropoietic cytokines have both pro- and anti-inflammatory characteristics and are major players in hematopoiesis, acute-phase responses, and immune responses.[181] Signal transduction includes activation of Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways and MAPK cascades.[181] Neuropoietic cytokines are expressed by a variety of cells types such as skeletal muscle, neurons, microglia, and astrocytes.

Most of the neuropoietic cytokines play a minor role in pain or inflammation. The neuropoietic cytokine IL-11 is primarily involved in hematopoiesis[182] and fertility.[183,184] OSM has been linked to sensory neuron development, and thus a deficiency in OSM has been shown to result in a marked decrease in reactions to thermal, mechanical, chemical, and visceral nociceptive stimulants.[185] The cytokines CLC and neuropoietin play prominent roles involving motor neuron development.[184,186,187] CLC is involved in astrocyte differentiation within the developing brain.[188] CT-1 is highly expressed in embryonic skeletal muscle and is predominantly involved with survival of motor neurons.[183] However, CT-1 was recently shown to induce IL-6 mRNA and protein expression in a time- and dose-dependent manner.[189]

As with almost all neuropoietic cytokines, IL-6 is important for differentiation, survival, and nerve regeneration. However, it plays a significant role in chronic pain. IL-6 represents a typical defense hormone involved in the activation of the immune and acute-phase responses.[182] IL-6 is synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts, and other cells in response to signals such as IL-1β, TNFα, and prostaglandins.[190–193] Centrally, IL-6 is known to be produced by neurons as well as astrocytes and microglia.[193–195]

Following peripheral axotomy, the presence of IL-6 mRNA is one of the earliest changes observed in the DRG and the brain.[196,197] IL-6 is produced both locally, at the site of peripheral nerve injury, and centrally, in response to nerve damage.[190] Following an injury, IL-6 mRNA and protein were primarily found in neurons; however, microglia and astrocytes are also known sources of IL-6 production.[190,198] Intraplantar injection of IL-6 into a rat induced dose-dependent mechanical hyperalgesia.[199] Within 3 hours after a sciatic nerve crush injury, IL-6 was produced both distally and proximally to the injured site.[200,201] Interestingly, a similar case was seen when the nerve was transected, indicating a source other than the neuronal body, such as macrophages or Schwann cells at or near the injury site were responsible for producing inflammatory cytokines.[200,201] Mechanical allodynia and up-regulation of IL-6 was observed in the sciatic nerve after 14 days following chronic constriction injury, crush injury, and axotomy.[202] After the sciatic nerve injury, IL-6 was found in both the ipsilateral and contralateral dorsal and ventral horns with the increase in IL-6 paralleling pain behaviors over time.[190,198,203] IL-6 is considered a pro-inflammatory cytokine; however, it has some anti-inflammatory characteristics as well. IL-6, LIF, and CNTF have been shown to inhibit TNFα expression.[204–208]

LIF and CNTF are very similar structurally and are both known to be important for motor neuron development.[183] However, LIF has been shown to increase mechanical hyperalgesia in a dose-dependent manner, whereas CNTF showed no effect.[209] There is basal expression of LIF in the PNS and following a nerve injury, LIF expression increases at the site of injury as well as within the DRG.[209] LIF is retrogradely transported to the DRG following application to a peripheral nerve or target tissue.[210,211] The LIF receptor is present on macrophages and may stimulate the release of mediators, such as the induction of substance P, vasoactive intestinal polypeptide, and galanin that modulate the excitability of sensory neurons.[209]

SUMMARY

Recent studies indicate that a communication exists between the immune and nervous systems. Although multiple conditions may generate neuropathic pain, a common underlying mechanism is the presence of inflammation at the site of the damaged or affected nerve(s). Microglia and astrocytes within the CNS have been shown to play a pivotal role in the development and maintenance of neuropathic pain. Following peripheral nociceptive activation (via nerve injury, infection, or inflammation), microglia become activated and release pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, thereby initiating the pain process. Microglia propagate the neuroinflammation by recruiting other microglia and eventually activating nearby

astrocytes, which prolongs the inflammatory state and leads to a chronic neuropathic pain condition.

Many substances have been identified as mediators in the neuropathic pain pathway. These mediators, such as cytokines and neurotrophic factors, have been shown to modulate pain by either direct interaction with neurons or by activating glial cells. Although numerous investigators have attempted to elucidate the role of neuroinflammatory agents with regard to pain, much conflicting evidence surrounds the effects induced by these mediators. In some instances, an agent may inhibit a pain pathway while serendipitously in another circumstance that same agent may promote a pain pathway. As such, more work needs to be done to better understand the pathways involved in the alterations in gene expression responsible for generating a chronic neuropathic pain condition.

A major component in the development of this neuroinflammatory response is the increased electrical afferent input into the dorsal horn that enhances the central sensitization process. Traditionally, most efforts in managing pain involve pharmacological intervention to modulate release of chemical neurotransmitters. As previously described, signal transmission within the nervous system employs both chemical and electrical pathways. Therefore, attenuation of these electrical signals via external electrical signals can be of therapeutic value.

A role of glial cells and the immune system has been established in the development and maintenance of chronic neuropathic pain. As activation of glial cells appears to be a pivotal component of the neuroinflammatory process, therapeutic measures attenuating nociceptive behaviors targeting these cells offer a prime area for future research.

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