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Role of glia in neuropathic pain

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1. ABSTRACT

Neuropathic pain is experienced as a result of disease or physical injury affecting the somatosensory system. It can be associated with abnormal sensations (dysesthesia) or evoked by normally nonpainful stimuli. Glia has emerged as key regulators of neuropathic pain perception and potential targets for drug development. Glia are activated upon peripheral nerve damage and secrete a number of proinflammatory factors. This process involves many mechanisms including neuroinflammation, ion channel activation, and ligand-receptor interactions. This review describes recent advances in the understanding of neuropathic pain, including the role of glia and their targeting by current treatment approaches.

2. INTRODUCTION

Neuropathic pain is caused by a lesion or diseases of the peripheral nervous systems (PNS) or central nervous systems (CNS), and is clinically characterized by spontaneous and evoked types of pain, which have distinct pathophysiological mechanisms (1-3). In some cases, a nerve lesion can induce molecular changes in nociceptive neurons, rendering them exceedingly sensitive and prone to aberrant spontaneous activity. Inflammatory reactions within the damaged nerve trunk cause the sensation of pain by inducing secondary changes in processing neurons in the spinal cord and brain (1, 4, 5). Recent studies have implicated glia in this process (6-8). Upon peripheral nerve injury, microglia are activated by the release of cytokines

including ATP, chemokine (C-C motif) ligand 2 (CCL2), CCL3, as well as Na^+, K^+, and Ca^{2+} (9, 10). Activated microglia secrete proinflammatory factors, which in turn activate astrocytes (11); such changes in central pain modulatory systems can lead to further hyperexcitability. Treatment approaches for neuropathic pain are still unsatisfactory, and recently, the concept of analyzing pain based on underlying mechanisms—specifically by targeting glia—has begun to emerge (4, 11, 12). A deeper understanding of the general mechanisms that generate the sensation and symptoms of pain can enable the identification of the precise underlying cause of pain in a patient. Combining this clinical characterization with an appropriate selection of drugs should make it possible to provide personalized treatments tailored to each individual (10, 13, 14).

3. CHARACTERISTICS OF NEUROPATHIC PAIN

Pain is an unpleasant feeling provoked by intense or damaging stimuli, which plays an important physiological role in reflexive self-defense (1, 3), motivating the avoidance or withdrawal from noxious surroundings, or protection of an injury during healing. Pain is also symptomatic of many medical conditions, and can significantly interfere with the quality of life and general functioning of an individual (15-18). Upon peripheral stimulation, pain information is transmitted by unmyelinated C fibers and thin myelinated Aδ fibers to the dorsal horn in the spinal cord (2, 12), where second order nociceptive neurons are activated by neurotransmitters released from primary afferents, such as glutamate and neuropeptides (*e.g.*, substance P and calcitonin gene-related peptide) (19-21). This information is relayed to the thalamus and then to the parietal lobe of cerebral cortex where the sensation of pain is registered. This type of pain is usually transitory, lasting only as long as the noxious stimulus or injury/pathology lasts; however, the pain can continue beyond the stimulus duration, developing into a chronic problem (22-24). Chronic pain does not convey any useful information; in such cases, thermal stimuli and painful pressure are amplified, and even light touch can be perceived as painful (conditions known as hyperalgesia and allodynia, respectively). Persistent pain can be inflammatory pain or neuropathic pain (25-27). For the former, tissue inflammation lowers the nociceptive excitation threshold, while for the latter, pain is perceived as a result of central or peripheral nervous system damage (28, 29).

Neuropathic pain can be categorized as peripheral, central, or mixed (peripheral and central), depending on the region of the nervous system that is affected (29, 30). In general, any one of these types can occur as a result of trauma, viral infection, medications, metabolic insults, or stroke (31-33). More specifically, in the CNS, pain can arise from spinal cord injury, multiple sclerosis, and stroke; common causes in the PNS are herpes zoster or HIV infection, diabetes, nutritional deficiency, toxins, immune disorders, and physical trauma to a nerve trunk, and they can also be remote manifestations of malignancies (34, 35). Pain is a common symptom in diabetes and cancer patients; in the latter, the cause can be direct injury to peripheral nerves from tumor growth, or a side effect of surgery, chemo- or radiotherapy (36, 37).

Inflammation in any part of the body is coupled with pain sensation by the release of proinflammatory factors, including prostaglandin E_2 (PGE_2) (38-40), tumor necrosis factor α (TNF-α) (41) and interleukin 1β (IL-1β) (42), as well as nerve growth factor (NGF) (43, 44) by non-neural and immune cells, which stimulates nociceptor terminals in the peripheral tissue and thereby increases pain sensitivity. Neuroinflammation in the CNS resulting from brain trauma, infection, and in neurodegenerative diseases is characterized by the activation of glial cells, specifically microglia and astrocytes, and can actually contribute to the development and progression of neurodegeneration (45, 46).

4. CHARACTERISTICS OF GLIA

Glia are the other major cell type in nervous system besides neurons, and comprise diverse, specialized cell types in the PNS (Schwann cells, satellite and perineural glia) and CNS (microglia, astrocytes, oligodendrocytes, and perivascular glia) (47, 48). Glia comprise ~70% of the total cell population in the nervous system, and can be broadly classified as micro- and macroglia. Microglia, which constitute 5–10% of the glial population, are macrophage-like cells. Macroglia can be neuroectoderm-derived oligodendrocytes, which produce myelin to enshealth neuronal axons, or astrocytes which are 40–50% of all glia and are therefore the most abundant glia type in terms of number and volume. Under normal conditions, microglia and astrocytes are quiescent; they are activated in response to injury or in disease states, and contribute to the pathogenesis of neurological disorders (49).

It has become increasingly evident that glia provide indispensable protection and support for neurons in the CNS and PNS (50, 51). There are few connective tissues in the nervous system; instead, glia form a network that physically support neurons but also maintain homeostasis in the brain, by insulating neurons from harmful agents in the surroundings, destroying pathogens, removing dead neurons, and healing injuries (52). Microglia are constantly engaged in repairing minor insults, and a failure in this process can lead to disease. In addition to these functions, glia are known to play an important role in the development of neuropathic pain (53, 54).

5. ROLE OF GLIA IN NEUROPATHIC PAIN

Neuropathic pain is a debilitating condition affecting millions of individuals worldwide. It has been nearly two decades since a role for glia in pain sensation was first proposed (55, 56); however, only recently has this relationship been conclusively demonstrated (57): peripheral nerve damage was followed by activation of microglia 24 h after nerve damage in the dorsal horn, while astrocyte activation was observed as late as 3 days after the injury. Microglia can remain activated for up to 3 months

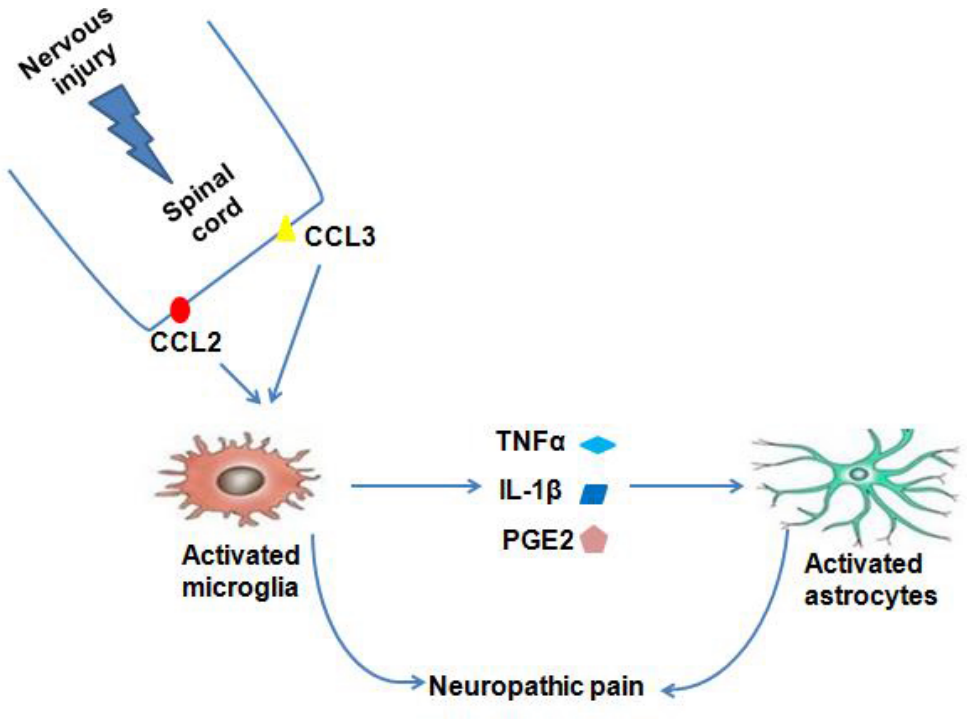

Figure 1. Peripheral nerve damage was followed by activation of microglia. Microglia are activated by injury released cytokines and proinflammatory factors, which induce the activation of astrocytes, a process involving various downstream signaling pathways, such as neuroinflammation.

before subsiding (3, 58), and have been associated with the onset of pain symptoms such as allodynia or hyperalgesia.

A mechanism underlying pain sensation is neuroinflammation at the site of injury (31, 46), which initiates a cascade of events including increased capillary permeability and local perfusion, the concentration and activation of innate immune cells at the site of injury, irritation, or infection (45). Neuroinflammation activates perivascular microglia and astrocytes located in the spinal cord and brain, which are important mediators of nociception (31, 59). Immunoactive substances released at the site of injury such as cytokines, chemokines, and cellular adhesion molecules act locally but also initiate a systemic immune response, by inducing the expression of surface antigens that enhance a CNS immune cascade, leading to the infiltration of immune cells at the site of injury (2, 60, 61); however, the glia-mediated release of inflammatory cytokines has been shown to promote neurodegeneration (62). Thus, early and delayed inflammatory responses mediated by microglia and astrocytes, respectively, can have both protective and adverse effects on the organism (60, 63).

5.1 Neuropathic pain induced by glia activation

5.1.1. Role of microglia in neuropathic pain

Microglia, which are derived from the transformation of macrophages or their monocyte precursors, are part of the immune system that protects the brain against infection and injury (64). Microglia have been implicated in diseases such as diabetic and post-herpetic neuropathy, viral infections, and autoimmune and neurodegenerative disorders (65). Microglia comprise as little as 5–20% of all glial cells under normal physiological conditions; they are the first cells to become activated by the release of ATP, CCL2 and 3, and Na^+, K^+, Ca^{2+} following peripheral nerve injury (Figure 1), and transmit the sensation of pain via unmyelinated C fibers and thin myelinated Aδ fibers to the dorsal horn of the spinal cord to astrocytes (12, 66).

Upon nerve injury, microglia are stimulated to proliferate and undergo profound morphological changes. New cell surface markers are expressed, and cells migrate to the site of injury, where they engage in phagocytosis and produce a variety of proinflammatory factors (67-69). Markers that are expressed include complement receptor 3, cluster of differentiation (CD) 11b, ionized calcium binding adaptor molecule 1, CD14, and Toll-like receptors, with concomitant activation of p38 mitogen-activated protein kinase (MAPK) (70-73). Activated microglia produce cytokines such as IL-1α and β, TNF-α, IL-6 and -12, fractalkine, and macrophage inflammatory protein 1α and β, as well as inducible nitric oxide synthase (iNOS) and free oxygen and nitrogen radicals. These factors, which can evoke allodynia and hyperalgesia, also play important roles in nociception (74, 75).

The P2 receptors expressed by microglia are divided into ionotropic (P2X) and metabotropic (P2Y) receptor subfamilies. P2X receptors (of which there are seven types, P2X1–7) are ATP-binding ion channels, while

P2Y receptors (of which there are eight types: P2Y1, 2, 4, 6, 11, 12, 13, and 14) are coupled to intracellular second messenger systems through heteromeric G proteins (76-78). Accumulating evidence points to P2 receptors of microglia as mediators of the pain response. P2X4R expression was significantly upregulated in microglia in the spinal cord upon peripheral nerve injury; the ensuing tactile allodynia was reversed by pharmacological blockade of P2X4R (70, 79). The release of brain-derived neurotrophic factor (BDNF) by P2X4 activation is necessary for the sensation of pain: P2X4R null mice have reduced levels of microglial BDNF, impaired BDNF signaling in the spinal cord, and fail to develop mechanical allodynia following peripheral nerve injury [78]. P2X7R and P2Y12R expression is upregulated in spinal microglia after peripheral nerve injury, and their inhibition can suppress microglia-mediated pain (78, 80, 81). Moreover, systemic administration of the selective P2X7R inhibitors (A-740003 and A-438079) reduced tactile allodynia in three different rat models of neuropathic pain (82), and A-438079 treatment reduced activity induced by innocuous stimuli in dorsal horn neurons in these animals. IL-1β released from LPS-treated microglia in the dorsal horn following ATP stimulation was dependent on P2X7Rs, which stimulated the release of cathepsin S, a lysosomal cysteine protease that contributes to the transmission of pain information (78, 81). In summary, microglia respond to injury or pathology in a stimulus-specific manner, which contributes to the perception of pain by the organism.

While microglia are involved in the initial stages of pain development, the sensation is sustained by astrocytes. Unlike microglia and oligodendrocytes, astrocytes form networks that are closely associated with neurons and blood vessels (83). Activated astrocytes were initially discovered in rats on the damaged side of the spinal cord following sciatic nerve injury (84). Like microglia, astrocytes in the CNS, particularly those in the spinal cord, undergo various phenotypic changes including changes in gene expression in response to inflammation resulting from peripheral nerve injury or tumor invasion (Fig.1); this includes increased expression of the glial fibrillary acidic protein, an astrocyte marker, and production of cytokines such as TNF-α and IL-1β (85). Activated astrocytes also secrete factors such as nitric oxide (NO), excitatory amino acids, prostaglandins, and ATP that mediate pain hypersensitization. Intrathecal administration of TNF-α and IL-1β antagonists or IL-6-neutralizing antibody has been shown to alleviate pain-induced behaviors in animal models (86-88).

Multiple lines of evidence suggest that astrocyte activation is sufficient to produce pain symptoms. Implantation of neural stem cells into the injured spinal cord of rats resulted in allodynia in the forepaws (89, 90), attributable to the differentiation of the stem cells into astrocytes; indeed hypersensitivity was prevented by first transfecting the stem cells with a vector expressing the neurogenic gene neurogenin-2 in order to suppress the generation of astrocytes. Moreover, transplantation of glial restricted precursor-derived astrocytes induced mechanical allodynia (91, 92). Similarly, intrathecal injection of TNF-α-activated astrocytes was sufficient to induce chronic pain in naïve animals through the stimulation of CCL2 release (93).

A major function of astrocytes is the uptake of extracellular glutamate and GABA in the synaptic region through astrocytic transporters such as glutamate transporter-1 and glutamate/aspartate transporter, along with cotransport of Na^+/H^+ and counter transport of K^+. Reduction in the expression and uptake activity of glutamate transporters plays an essential role in both the induction and persistence of pain following peripheral nerve injury and in taxol-induced hyperalgesia (94, 95). Glutamate transporter expression is consistently downregulated in spinal models of pathological pain; conversely, their increased expression or function can prevent pain from developing (96, 97). For example, the drug propentofylline has protective effects against post-ischemic damage in the CNS by potent dose-dependent induction of glutamate transporter-1 mRNA and protein expression, accompanied by inhibition of CCL2 release. Conversely, injection of the glutamate uptake blocker threo-beta-benzyloxyaspartate leads to the manifestation of spontaneous nociceptive behavior (96, 97).

Ca^{2+} signaling also plays an important role in the sensation of pain. In the gap junction-coupled networks formed by astrocytes, Ca^{2+} signals are transmitted in the form of oscillations. The major structural components of gap junctions are connexins (Cx); in the mammalian nervous system, at least six family members have been identified (Cx26, 29, 30, 32, 36, and 43) (98). Cx30 and 43 are specifically expressed by astrocytes, and Cx43 expression is significantly upregulated in response to facial nerve lesions, spinal cord injury, and inflammation induced by complete Freund's adjuvant (99). Inhibiting gap junction function by application of the nonselective inhibitor carbenoxolone was shown to produce analgesia in different pain models (100). In addition, intrathecal injection of carbenoxolone in rats reduced mechanical allodynia caused by sciatic nerve inflammation in the contralateral paw, implicating gap junctions of the astrocyte network in the spread of pain beyond the injury site.

5.2. Molecular basis of neuropathic pain by glia activation

Nociceptors are normally silent and respond to potentially noxious stimuli. These sensory neurons become abnormally sensitive and develop aberrant spontaneous activity upon peripheral nerve damage, resulting in changes at the molecular and cellular levels. Pain information is transmitted by unmyelinated C and thin myelinated Aδ fibers to the dorsal horn; microglia are then led to the site of injury by secreted CCL2 and CCL3 (71, 101), and in turn activate astrocytes. Both types of glia activate p38 MAPK to induce the synthesis and release of PGE2, IL-1β, IL-6, TNF-α, and NO (102-104). Such proinflammatory factors stimulate nociceptor terminals in the peripheral tissue, thereby increasing pain sensitivity, neurotoxicity, and chronic inflammation (105); indeed, inhibition of p38 in the spinal cord has been shown to attenuate

postoperative pain (102-104). Thus, glia provide the molecular link between inflammation and neuropathic pain.

Ectopic spontaneous activity following nerve injury is correlated with increased transcript expression of voltage-gated sodium channel genes in primary afferent neurons (106, 107). Genes encoding the voltage-gated sodium channels $Na_v1.8$ and $Na_v1.9$ are specifically expressed in nociceptive primary afferent neurons, indicating that sodium channels are an important mediator of pain responses; this is supported by the fact that lidocaine, a sodium channel blocker, produces pain relief (106, 107). The clustering of sodium channels in glia could be responsible for the lowering of the action potential threshold and the consequent hyperactivity that is observed.

The expression of other receptor types—some of which are expressed only at low levels under normal conditions—is also upregulated in glia upon damage to peripheral nerves. For example, vanilloid receptors (TRPV1) in nociceptive afferent fibers are activated by nerve injury (108, 109). TRPV1-deficient mice fail to develop hyperalgesia following tissue inflammation, providing evidence for the contribution of TRPV1 to the development of C-nociceptor sensitization and hyperalgesia (110).

5.3. Glia-targeted treatments for neuropathic pain

The management of pain resulting from lesions or disease in the nervous system represents a significant clinical challenge. Until recently, the treatment of neuropathic pain has been largely neglected (111) and has aimed to provide general pain relief without addressing specific etiologies, which is the likely reason for the limited success in outcomes for patients. With the current understanding of the role of glia in pain transmission, attention is focused on the development of therapies that target activated glia. For example, studies have examined the role of purinergic P2XRs/P2YRs expressed by spinal microglia upon peripheral nerve injury. Since P2XRs/P2YRs-mediated activity contributes to the pathological enhancement of pain information processing in the dorsal horn, a predicted therapeutic benefit of interfering with microglial P2XRs and P2YRs is the recovery of normal pain sensitivity, since these molecules are only upregulated in activated microglia in the spinal cord that receives projections from damaged sensory fibers (68).

In addition, recent studies have shown that glia-specific inhibitors such as propentopfline and pentoxifylline suppress secretion of various cytokines, thus preventing the development of pain (112-114) in both animal models and clinical trials (115, 116). Minocycline is another potent inhibitor of microglial activation that suppresses proliferation as well as the activity of matrix metalloproteinases (MMP) 2 and MMP9 (117). Clinical data demonstrate that minocycline can penetrate the blood-brain barrier and affords protection in neurodegenerative diseases that are associated with microglial activation, by reducing levels of proinflammatory factors, such as TNF-α, IL-1β, IL-6, iNOS, and cyclooxygenase-2 (118, 119). Both peripheral and intrathecal administration of minocycline have been shown to reduce pain symptoms as allodynia and hyperalgesia (119).

6. CONCLUSIONS

Neuropathic pain syndromes are chronic pain disorders caused by lesions or disease in parts of the nervous system that normally transmit pain information. Recent research has focused on the role of glia in the development of pain. Microglia activated by injury release cytokines and proinflammatory factors, which induce the activation of astrocytes, a process involving various downstream signaling pathways. Therefore, interfering with the function of activated glia is a promising treatment strategy that affords many potential targets for drug development. To this end, glia inhibitors have been shown to slow the development of pain and facilitate the recovery of motor functions following nerve damage. However, more work needs to be done to elucidate the diverse molecular mechanisms underlying neuropathic pain so as to enable the development of individualized and clinically effective treatments.

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Abbreviations: BDNF, brain-derived neurotrophic factor; CCL, chemokine (C-C motif) ligand; CD, cluster of differentiation; Cx, connexins; CNS, central nervous systems; IL, interleukin; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinases; NGF, nerve growth factor; NO, nitric oxide; PGE2, prostaglandin E2; PNS, peripheral

nervous systems; TNF-α, tumor necrosis factor α; TRPV1, vanilloid receptors

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