REVIEW ARTICLE

ANAESTHESIA, PAIN & INTENSIVE CARE
www.apicareonline.com

# Biomarkers for microglia activation in neuropathic pain

Putu Eka Widyadharma[1], Aurelia Vania[2], Jimmy FA Barus[2], Yudiyanta[3], Thomas Eko Purwata[1]

[1]Department of Neurology, Faculty of Medicine, Udayana University and Sanglah General Hospital, Bali, (Indonesia)
[2]Department of Neurology, School of Medicine and Health Sciences, Atma Jaya Catholic University, Jakarta, (Indonesia)
[3]Department of Neurology, Faculty of Medicine, Public Health, and Nursing, Gadjah Mada University, Yogyakarta, (Indonesia)
**Correspondence**: Putu Eka Widyadharma, Department of Neurology, Sanglah General Hospital, Diponegoro Street, Denpasar, Bali 80113, (Indonesia);
E-mail: eka.widyadharma@unud.ac.id
**Received:** 12 June 2019, February 2020,
**Accepted:** 2 March 2020

## ABSTRACT

Neuropathic pain (NP) is a result of direct disturbances of somatosensory pathways. Its pathophysiology includes various mechanisms. Recent studies have reported an important role of microglia in the NP mechanism. There are several chemical molecules which are involved in microglia activation. The activated microglia will, in turn, enhance some receptors expression that can be used as markers of its activation. Though we still need future studies about precise microglia role in NP mechanism, the chemical mediators that initiate microglia activation and the alteration of some receptors in the activated microglia which have been found from previous studies can be the interesting future research materials and the promising target for a new therapy for NP.

**Key words:** Neuropathic pain; Microglia activation; Biomarker

**Citation:** Widyadharma PE, Vania A, Barus JFA, Yudiyanta, Purwata TE. Biomarkers for microglia activation in neuropathic pain. Anaesth pain intensive care 2020;24(1):87-93. DOI: https://doi.org/10.35975/apic.v24i1.1229

## INTRODUCTION

Neuropathic pain (NP) was defined by IASP as "pain emerging as a direct consequence of a lesion or a disease of the somatosensory system".[1] NP is commonly characterized by electric-shock-like sensation, burning sensation, hot or cold pain, spontaneous ongoing pain, paresthesia or allodynia. If the symptoms persist, NP has a tendency to become a chronic pain. The prevalence of chronic pain caused by NP has been reported to be in the range of 7-10%.[2]

NP does not occur through the same mechanism with other conditions that lead to chronic pain caused by inflammatory pain. The primary cause of the latter condition is an inflammatory process with chemical mediators alteration at the site of injury.[2] Lesions of nervous system which lead to NP most commonly involve nociceptive pathways.[1] Disturbances of the somatosensory system can induce abnormal changes in transmission of sensory signals from peripheral system to spinal cord and brain.[2] NP includes some complex mechanisms. Some different mechanisms could appear in one patient with NP, and these different mechanisms can cause similar symptoms. At this time, most of analgesic drugs do not completely eliminate symptoms of NP. The management of NP is a real challenge and it drives researchers to learn more about the mechanism and more appropriate therapy for NP. Therapeutic approach with the target based on the mechanism of pain in one patient is the most important thing to get efficient analgesic therapy.

NP is caused by peripheral and central mechanisms. Peripheral mechanism includes ectopic discharges in lesioned fibers and their corresponding ganglia, abnormal activity in axons undamaged by the lesion, alterations in the expression and regulation of intracellular calcium ion and modulatory receptors on primary afferent terminals, neuro-immune interactions resulting in enhanced and/or altered production of inflammatory signaling molecules, sensory-sympathetic coupling and other alterations in receptor signaling. Continuous activities from sensory peripheral system cause the changes in sensory central nervous system that include alteration of presynaptic and postsynaptic molecules, dysfunction

of inhibitory interneuron and descendent inhibition system. Finally, all of these alterations result in central sensitization.[1,2]

Inflammation in the peripheral or central systems has been known to have a contribution to ectopic neuron activity and central sensitization.[1] Recent studies have found some evidence of a vital role of microglia as a central nervous system immune cell in NP mechanism.[1,3-7] These findings promote more studies for new NP therapeutic approach with microglia activation as the target. This article has been written to learn more about chemical mediators that have roles as the initiation factors or markers of microglia activation and some pathways that contribute to cause NP after microglia activation has occurred. These biochemical markers could be expected to be a new target for NP therapy or as a new tool to monitor the therapy that inhibits microglia activation.

## ROLE OF MICROGLIA CELL IN NP

Microglia are macrophage-like cells in the central nervous system that regulate homeostasis in the brain and spinal cord. These immune cells are emerging as the key to the pathogenesis of some neurodegenerative diseases. These cells contribute to the pathogenesis of the disease via neuroinflammatory reactions.[6] Lesion in both peripheral and central system will trigger the inflammatory reaction. Although neuroinflammatory reaction has neuroprotective and neurotrophic effects, it is also associated with the nerve damage that causes NP. Microglia are the first immune cells that are activated in dorsalis root ganglion after nerve injury.[1]

Microglia activation can be induced by several chemical mediators that are released from neurons after nerve injury, such as colony stimulating factor-1, fractalkine, chemokine (C-C motif) ligand 2 (CCL2), neuregulin-1, and matrix metalloproteinase (MMP).[6] The activated microglia will proliferate and change its morphology from "resting" state to "amoeboid" state. This activation will also increase microglia markers expression (for example CD11b, Iba1, P2X4, CX3CR1, TLR4).[7,9] Microglia will also migrate to the site of injury and stimulate the production of proinflammatory cytokines such as IL-6, IL-1β, TNFα, colony stimulating factor-1 (CSF1), nitric oxide, brain-derived neurotrophic factor (BDNF), excitatory amino acids, ATP, and prostaglandins.[1,3,6] These inflammatory mediators are the key to the occurrence of NP.[6] Activated microglia will increase nerve excitation and decrease the inhibition by interneurons.

Activation of microglia occurs during the early phase of NP and before astrogliosis. It supports the hypothesis that microglia could be important for the initiation of NP, whereas astrocytes are essential for the alteration of acute pain to chronic pain.[9] One of the studies in animal has found that microglia activation will cause spinal hyperexcitability and is associated with pain and allodynia conditions. That inhibiting microglia activation diminished allodynia and hypersensitivity after nerve injury has been reported in several studies.[3]

## BIOCHEMICAL MARKERS IN THE PROCESS OF MICROGLIA ACTIVATION

### A. Neuregulin-1 dan erbB2

Neuregulin-1 is a family member of growth factors released after nerve injury and involved in neuronal development. It is one of the important mediators for microglia activation. Calvo et al. found the release of neuregulin-1 from primary afferent within dorsal horn following spinal nerve ligation in mice and it activated erbB2 receptor. Receptors of neuregulin-1 including erbB 2, 3, and 4 are found on the surface of microglia cells, suggesting that there is a neuron-glial interaction. Calvo et al. also reported that activation of erbB2 by neuregulin-1 will stimulate MEK/ERK pathway in microglia. MEK/ERK activation will drive proliferation of microglia cells and the release of inflammatory mediators, and contribute to the development of NP. Some studies have shown that inhibition of neuregulin-1, erbB2, MEK/ERK pathway will reduce microglial proliferation as well as mechanical and cold pain related hypersensitivity. These studies proposed that neuregulin-1 and erbB2 could be the potential target of NP therapy.[3,10,11]

### B. Matrix Metalloproteinase

The MMPs are a family of zinc-containing endoproteinases that share structural domains but differ in substrate specificity, cellular sources, and inducibility. The major function of MMPs is the degradation and remodeling of extracellular matrix component. MMPs are synthesized in their inactive proform, then activated extracellularly by proteolytic cleavage that is regulated by several inflammatory mediators, such as cytokines, chemokines, free radicals, and steroid. As proteolytic enzymes, MMPs take part in the development and physiological activities of central nervous system, such as myelin formation, axonal growth, angiogenesis, and regeneration. In general, if there is an abnormal expression or overproduction of MMPs, they will cause tissue destruction.[12]

One of the variants of MMPs, MMP-9, play a role in microglia activation. Some studies have reported there is an increased of MMP-9 in dorsalis ganglion following nerve injury. A study from Kawasaki et al. proved this hypothesis. They found a transient and rapid upregulation of MMP-9 with its peak at day 1

and started to decline after three days in dorsal root ganglion (DRG) primary sensory neuron after spinal nerve ligation in mice.[13] Liou et al. also found rapid increased MMP-9 expression in peripheral nerve and spinal cord after partial sciatic nerve ligation in mice.[14] These studies concluded that MMP-9 is involved in starting the NP rather than maintenance of NP.[3,5]

MMP-2, the other variant of MMPs, has also been found to be associated with NP mechanism. Unlike MMP-9, MMP-2 has more important role in maintenance of NP than initiation of NP. Kawasaki et al. showed upregulation of MMP-2 occurred at day seven and was still detected on day 21. They concluded that MMP-9 and MMP-2 take part in NP through IL-1β cleavage and microglia activation.[13] In one study, MMP-2 intrathecal injection lead to NP condition in mice and peritoneal administration of MMP inhibitor, GM6001, diminished thermal hyperalgesia and allodynia triggered by nerve injury. Therefore, either MMP-2 or MMP-9 were considered as important chemicals for the development and maintenance of NP, as they play a role in the initiation and persistence of NP.[3]

## C. CCL2 and Chemotactic Cytokine Receptor-2

CCL2, also known as monocyte chemoattractant protein 1 (MCP1), will be upregulated in dorsalis ganglion after nerve injury. CCL2 can induce microglia activation.[15] CCL2 has low expression in normal condition, but it will increase its level after nerve injury. CCL2 binds to the chemotactic cytokine receptor-2 (CCR2) which is a G-protein coupled receptor in microglia cells. Zhang et al. using partial sciatic nerve ligation in mice model showed that CCR2 knocked out mice had reduced microglia activation.[16] Zhu et al. study using a rat model with lumbar disk herniation reported that there was an increased CCR2 expression in DRG and spinal cord. They also found that intratechal injection of CCR2 inhibitor could diminish pain hypersensitivity induced by NP.[17]

Thacker et al. found an increased CCL2 concentration at day 1 in DRG after chronic constriction injury and spinal nerve ligation in rats.[18] White et al. in their study using chronic compression of DRG in mice reported that CCL2 will cause neuron depolarization, trigger excitatory condition in neuron of dorsalis ganglion that will result in NP symptoms such as allodynia.[19] The results of several studies have found that intrathecal injection of CCL2 would result in allodynia condition in 24 hours, while injection of CCL2 neutralizing antibody or CCR2 antagonist would prevent allodynia. These studies concluded CCL2 and NP interaction occurs by neuron excitation and microglia activation.[3] CCL2, however, also has direct effects on neurons in dorsal root ganglion demonstrating that the role of this chemokine might be various and not only limited to the microglia activation.[10]

## D. P2X4 receptor

P2X4 receptor (P2X4r) is belonged to purinergic system that has intrinsic pores that open on binding of extracellular ATP.[5] P2X4r is exclusively expressed in activated microglia after peripheral nerve injury.[1,4,5] P2X4r expression upregulation in microglia is known to have a positive interaction with pain hypersensitivity. Some studies have shown that the blockage of P2X4 receptor would attenuate allodynia after nerve lesion, while injection of microglia with P2X4r expression would trigger allodynia in naïve animal.[1,9] Role of P2X4r was first known in 2003. In that year, Tsuda et al. published a report that described an increased expression of P2X4r in microglia cells that have been activated after spinal nerve ligation in rats. They also reported that intrathecal administration of P2X4r inhibitor reduced tactile allodynia and intrathecal administration of P2X4r-stimulated microglia was sufficient enough to develop tactile allodynia in normal rats.[20] Their study result was supported by Ulmann et al. who also found upregulation of P2X4r in microglia after partial sciatic nerve ligation in mice and P2X4r knock out mice were more resistant to tactile allodynia induced by peripheral nerve injury.[21]

Activated P2X4r will increase the release of BDNF via calcium ion influx and activation of P38 mitogen-activated kinase, and it also increases synthesis and SNARE-mediated exocytosis of BDNF. BDNF causes disinhibition in the dorsalis horn through increasing level of intracellular chloride by downregulating KCC2 function as neuronal chloride transporter.[3,5,22-23] This process triggered by BDNF would change the GABA and glycine effects to be neurons depolarization rather than hyperpolarization.[4,5]

At last, activated P2X4r will result in increasing of neuron discharge, response to non-nociceptive stimuli, and spontaneous neuron activity.[23] The activation of P2X4r itself is mediated by upregulated IRF8-IRF5 cascade and chemical mediators released by the damaged neurons, such as chemokine, fractalkine, CCL2, and CCL21.[22,24] The result from several studies reported P2X4r has a crucial role for microglia to be an important cause of NP mechanism.

## E. Toll-Like Receptor-4

Toll-Like Receptor-4 (TLR4) involved in innate immune system is mainly expressed in microglia.[8] Activated microglia will increase TLR4 expression on its surface. TLR4 is a transmembrane receptor protein receptor with extracellular leucine-rich repeat domains and cytoplasmic repeat domains.[15] Lehnardt et al. reported that TLR4 is expressed exclusively in microglia.[25] Some microglia markers,

including TLR4, can be used to detect when the microglia activation is initiated and the duration of microglia activation during the episode of NP. Tanga et al. used real-time PCR identification to detect CD14 and TLR4 in their research of microglia activation, and found the highest peak at day four and a return to normal level by day 28 after spinal nerve transection in rats.[26] While Jurga et al. found enhanced expression of TLR4 in spinal medulla and dorsal ganglion at day seven and day 14 after chronic constriction injury in rats in their study.[27] Tanga et al. in their later study using spinal nerve transection model in mice reported TLR4 antisense ODN could reduce pain hypersensitivity, microglial activation, and production of proinflammatory cytokines.[28]

Recent evidence indicated essential role of microglia with TLR2 and TLR4 expression in NP mechanism. Neuronal damaged can lead to the release of proinflammatory factors that activate spinal microglia, one of the pathways is via TLR4/NF-κB signaling pathway. Studies reported the decreasing microglia activation, reduction of cytokines production, increasing pain resistance, and decreasing pain hypersensitivity in TLR4 deficient animals.[15,27,29] Diminished mechanical allodynia and thermal hyperalgesia found in TLR4 knockout mice were showed in Bettoni et al. study using chronic constrictive injury model.[29]

Jurga et al. found the administration of LPS-RS (TLR2 and TLR4 antagonists) and LPS-RS ultrapure (TLR4 antagonist) could diminish pain after chronic constriction injury, in which they used mice with nerve injury-induced NP model. These TLR antagonists enhanced buprenorphine activity as antiallodynia and antihyperalgesia, but they did not show the same effect in morphine.[27] Previous studies reported important contribution of TLR4 in initiation and maintenance of microglia activation and NP.

## F. Fractalkine and CX3CR1 Receptor

Fractalkine (FKN) is expressed primarily in neuron of the central nervous system. The expression of FKN can also be observed in the cell body of peripheral sensory neurons in dorsalis ganglion. FKN is a neuron transmembrane glycoprotein and can be cleaved from neuron as a result of increased neuronal activity.[3,30] FKN can be cleaved by the protease Cathepsin S which is released by activated microglia.[3,15] The cleaved FKN binds to CX3CR1 receptor in microglia, creating a positive feedback loop for the activation of microglia cells.[3] CX3CR1 is seven-transmembrane domained G-protein coupled receptor and is expressed abundantly in both blood leucocyte cells and microglia. Upregulation of CX3CR1 occurs in spinal microglia following injury to the peripheral nerve.[30] Level of FKN and Capthesin S activity in cerebrospinal fluid increased significantly after peripheral nerve injury, associated with microglia activation.[15]

Several studies reported that there is increased FKN concentration and role of CX3CR1 expression in NP occurrence after nerve injury.[3,9,30] Zhuang et al. found there was an upregulation of CX3CR1 in microglia that started at day 1 and reduction of mechanical allodynia after injection of neutralizing antibody of CX3CR1 in mice after spinal nerve ligation.[31] Thermal hyperalgesia and mechanical allodynia were attenuated in CX3CR1 knockout mice, compared to naïve mice after partial sciatic nerve ligation. This finding was showed in a study by Staniland et al.[32] While Hu et al. reported that there were delayed appearance of mechanical allodynia, depressed microglia activation, and inhibition of p38MAPK expression after intrathecal administration of CX3CR1 neutralizing antibody in rats with bone cancer pain model.[33]

FKN and CX3CR1 activation result in phosphorylation of p38MAPK, which subsequently stimulate the release of inflammatory mediators such as IL-1β, IL-6 and NO in microglia that are involved in increasing pain sensation.[3,30] The pro-nociceptive effects of the CatS/FKN/CX3CR1 pathway are crucial for the maintenance phase of NP. Inhibition of CX3CR1, p38MAPK, dan Cathepsin S could diminish hypersensitivity condition in chronic pain model.[3,30]

## G. CD11b and Iba1

CD11b and Iba1 have been used most frequently for the study of microglia activation. CD11b is recognized by using OX-42 antibody. Upregulation of OX-42 or Iba1 are the marker of microglia activation.

CD11 is a family member of integrin and is expressed highly in monocytes or macrophages, common dendritic cells, and microglia cells. CD11b in common dendritic cells and macrophages could inhibit T-cells activation and inflammatory process. Whereas CD11 in microglia and its role in NP mechanism has not been known.[34] In the pain model that did not involve nerve injury, OX-42 expression did not increase significantly. Nerve injury, for example, lesion in sciatic nerve, caused enhancing of OX-42 expression.[7] Mika et al. used C1q and OX-42 antibody for binding CD11b to study about microglia activation and found that the microglia activation occurred on the second day after nerve injury, and reached its peak on day 7-9. They found the reduction of NP symptoms and microglia activation on day 17-21.[3,35] Clark et al. also found increased CD11b expression following microglia activation after partial sciatic nerve ligation in mice.[36] Although CD11b is a marker of microglia activation, the pain condition itself may not be related to CD11b changes in some cases. The increased CD11b expression could be more related to

nerve injury rather than the pain symptoms.[7]

Ionized calcium-binding adapter molecule-1 (Iba1) is a protein bounded to calcium ion and is expressed exclusively in microglia in the brain. Iba1 is one of the important keys for the migration and phagocyte activity of microglia. Anti Iba1 antibody is used to detect microglia in dorsalis ganglion. Romero-Sandoval et al. has used Iba1 as microglia marker and found that microglia is important for maintenance of neuropathic process.[37] Leinders et al. found evidence for an increased Iba1 expression in microglia that could be detected for at least 14 weeks after spinal nerve transection in rats.[38] But, the other study has reported that this marker was not enough to trigger hypersensitivity after microglia activation. These have been shown from a study using the mutant mice without P2X4r, whereas increasing of Iba1 and other morphology changes were not different from other normal mice, mechanical hypersensitivity did not occurred in mice without P2X4r.[23]

## FUTURE THERAPEUTIC IMPLICATIONS

There are various pathways occurring before and after microglia activation in NP mechanism. Which pathways is the core mechanism of neuropathic pain remains to be determined. Nevertheless, these findings of the crucial role of microglia have been interesting many researchers to find new therapeutic drugs for NP. Some researches have been done to know the effectiveness of some drugs associated with microglia activity.

Recently, minocycline, an antibiotic that has been used for a long time, is being studied as a future potential NP therapy. Rojewska et al. reported that minocycline could reduce MMP-2 and MMP-9 level in spinal cord and DRG. They administered minocycline repeatedly and via intraperitoneal pathway to mice before injury occurred. In their study, minocycline caused the change in the ratio of pronociceptive and antinociceptive factors.[39] Minocycline has also been studied in human in some studies, but the results of these studies have not shown a consistent effect of minocycline. Sumitani et al. in their study concluded that minocycline was not succeeded to decrease pain intensity, but it improved the affective disorders associated with NP.[40] Based on previous research, minocycline is known to have the ability to prevent the development of NP, but it is not effective to reduce NP after it occurs.[6] Therefore, we still need to do more research about minocycline as the NP preventive and therapeutic drug.

There are several chemicals that have been studied to target pathway involved in microglia activation. Japanese researches have been used NP-1815-PX compound as potential P2X4r antagonist. Intrathecal administration of NP-1815-PX could reduce mechanical allodynia induced by herpetic pain in a mouse model.[41] Yamashita et al. investigated duloxetine effect and found that duloxetine, one of the SNRI inhibitor agents, could inhibit recombinant P2X4r that they got from cultured cells. Intrathecal administration of duloxetine could also reverse mechanical allodynia by inhibiting microglia P2X4r in mice and rat.[42] Recently, there is another potent P2X4r antagonist that is being studied in Japan.[24] Hewitt et al. studied MIV-247, selective cathepsin S inhibitor, and reported that MIV-247 could attenuate allodynia and enhance antiallodynic properties of pregabalin and gabapentin without increasing the side effects of these drugs in mice model of NP.[43] Naltrexone and naloxone were shown as potential inhibitor of microglia activation and TLR-4 signaling pathway. They reduced production of ROS and phagocytosis induced by microglia activation.[44]

The results of microglia modulators in human still shows inconsistent effects that may be due to the different underlying cellular mechanisms in each NP cases or due to the study design. There were some other approaches that have been studied such as neuromodulation or stem cell therapy.[6] There are various chemical molecules involved in microglia activation and NP mechanisms. Effective analgesic drugs should target all these various mediators.

## CONCLUSION

Microglia have been emerging as an essential role in the pathophysiology of NP. Various receptor and inflammatory mediators are involved in microglia activation in the pathophysiology of NP. Both the receptor and their chemical mediators can be the useful biochemical markers for the future study about the pathophysiology of microglia activation and its relationship with the mechanism and progressivity of NP. Although we need to learn more, the studies which have been done before about microglia activation and biochemical markers involved in it can be the basis of the development of new therapy for NP.

**Declarations of interest**

We declare no competing interests.

**Conflict of interest:** None declared by the author

Authors' contribution:

IPEW: Corresponding Author, Concept, Manuscript Writing

AV, JFAB: Manuscript Writing

Y, THP: Manuscript Editing

## REFERENCES


1. Garcia-Larrea L. 2014. Pathophysiology of neuropathic pain: critical review of models and mechanism. In (Raja SN & Sommer CL, eds). Pain 2014 Refresher Courses: 15th World Congress on Pain. Washington: IASP Press, p 453-476.
2. *Colloca* L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yamitsky D, et al. *Neuropathic pain*. Nat Rev Dis *Primers.* 2017 Feb;16(3):17002. [PubMed] [Free full text] DOI: 10.1038/nrdp.2017.2
3. Zhao H, Alam A, Chen Q, Eusman MA, Pal A, Eguchi S, et al. The role of microglia in the pathobiology of neuropathic pain development: What do we know?. Br J Anaesth. 2017;118(4):504–516. [PubMed] [Free full text] DOI: 10.1093/bja/aex006
4. Alles SRA, Smith PA. Etiology and pharmacology of neuropathic pain. Pharmacol Rev. 2018;70(2):315–347. [PubMed] [Free full text] DOI: 10.1124/pr.117.014399
5. Tsuda M. Microglia in the spinal cord and neuropathic pain. J Diabetes Investig. 2016 Jan;7(1):17–26. [PubMed] [Free full text] DOI: 10.1111/jdi.12379
6. Cen G, Zhang YQ, Qadri YJ, Serhan CN, Ji RR. Microglia in pain: Detrimental and protective roles in pathogenesis and resolution of pain. Neuron. 2018 Dec 19;100(6):1292-1311. [PubMed] [Free full text] DOI: 10.1016/j.neuron.2018.11.009
7. Suter MR, Wen YR, Decosterd I, Ji RR. Do glial cells control pain? Neuron Glia Biol. 2007 Aug;3(3):255–268. [PubMed] [Free full text] DOI: 10.1017/S1740925X08000100
8. Campbell JN, Meyer RA. Mechanisms of neuropathic pain. Neuron. 2006 Oct 5;52(1):77–92. [PubMed] [Free full text] DOI: 10.1016/j.neuron.2006.09.021
9. Zhuo M, Wu GX, Wu LJ. Neuronal and microglial mechanisms of neuropathic pain. Mol Brain. 2011 Jul 30;4:31. [PubMed] [Free full text] DOI: 10.1186/1756-6606-4-31
10. Calvo M, Bennett DL. The mechanisms of microgliosis and pain following peripheral nerve injury. Exp Neurol. 2012 Apr;234(2):271–282. [PubMed] DOI: 10.1016/j.expneurol.2011.08.018
11. Calvo M, Zhu N, Grist J, Ma Z, Loeb JA, Bennett DL. Following nerve injury neuregulin-1 drives microglial proliferation and neuropathic pain via the MEK/ERK pathway. Glia. 2011 Apr;59(4):554–568. [PubMed] [Free full text] DOI: 10.1002/glia.21124
12. Könnecke H, Bechmann I. The role of microglia and matrix metalloproteinases involvement in neuroinflammation and gliomas. Clin dev immunol. 2013;2013:914104. [PubMed] [Free full text] DOI:10.1155/2013/914104
13. Kawasaki Y, Xu ZZ, Wang X, Park JY, Zhuang ZY, Tan PH, et al. Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. Nat Med. 2008 Mar;14(3):331–336. [PubMed] [Free full text] DOI: 10.1038/nm1723
14. Liou JT, Sum DC, Liu FC, Mao CC, Lai YS, Day YJ. Spatial and temporal analysis of nociception-related spinal cord matrix metalloproteinase expression in a murine neuropathic pain model. J Chin Med Assoc. 2013 Apr;76(4):201–210. [PubMed] [Free full text] DOI: 10.1016/j.jcma.2012.12.011
15. Smith HS. Activated microglia in nocicpetion. Pain Physician. 2010 May-Jun;13(3):295-304. [PubMed] [Free full text]
16. Zhang J, Shi XQ, Echeverry S, Mogil JS, De Koninck Y, Rivest S. Expression of CCR2 in both resident and bone marrow-derived microglia plays a critical role in neuropathic pain. J Neurosci. 2007 Nov 7;27(45):12396–12406. [PubMed] [Free full text] DOI: 10.1523/JNEUROSCI.3016-07.2007
17. Zhu X, Cao S, Zhu MD, Liu JQ, Chen JJ, Gao YJ. Contribution of chemokine CCL2/CCR2 signaling in the dorsal root ganglion and spinal cord to the maintenance of neuropathic pain in a rat model of lumbar disc herniation. J Pain. 2014 May 5;15(5):516-526. [PubMed] DOI: 10.1016/j.jpain.2014.01.49
18. Thacker MA, Clark AK, Bishop T, Grist J, Yip PK, Moon LD, et al. CCL2 is a key mediator of microglia activation in neuropathic pain states. Eur J Pain. 2009 Mar;13(3):263–272. [PubMed] DOI: 10.1016/j.ejpain.2008.04.017
19. White FA, Sun J, Waters SM, Ma C, Ren D, RipschM, et al. Excitatory monocyte chemoattractant protein-1 signalling is up-regulated in sensory neurons after chronic compression of the dorsal root ganglion. Proc Natl Acad Sci USA. 2005 Sep 27;102(39):14092–14097. [PubMed] [Free full text] DOI: 10.1073/pnas.0503496102
20. Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, et al. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature. 2004 Aug 14;424(6950):778–783. [PubMed] DOI: 10.1038/nature01786
21. Ulmann L, Hatcher JP, Hughes JP, Chaumont S, Green PJ, Conquet F, et al. Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. J Neurosci. 2008 Oct 29;28(4):11263–11268. [PubMed] [Free full text] DOI: 10.1523/JNEUROSCI.2308-08.2008
22. Tsuda M, Masuda T, Tozaki-Saitoh H, Inoue K. P2X4 receptors and neuropathic pain. Front Cell Neurosci. 2013 Oct 28;7:191. [PubMed] [Free full text] DOI: 10.3389/fncel.2013.00191
23. Beggs S, Salter MW. Microglia-Neuronal signalling in neuropathic pain hypersensitivity 2.0. Curr Opin Neurobiol. 2010 Aug;20(4):474–480. [PubMed] [Free full text] DOI: 10.1016/j.conb.2010.08.005
24. Inoue K, Tsuda M. Microglia in neuropathic pain: Cellular and molecular mechanisms and therapeutic potential. Nat Rev Neurosci. 2018 Mar;19(3):138–152. [PubMed] DOI: 10.1038/nrn.2018.2
25. Lehnardt S, Lachance C, Patrizi S, Lefebvre S, Follet PL, et al. The Toll-

Like Receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. J Neurosci. 2002 Apr;22(7):2478–2486. [PubMed] [Free full text] DOI: 20026268

26. Tanga FY, Raghavendra V, DeLeo JA. Quantitative real-time RT-PCR assessment of spinal microglial and astrocytic activation markers in a rat model of neuropathic pain. Neurochem Int. 2004 Jul-Aug;45(2):397–407. [PubMed] DOI: 10.1016/j.neuint.2003.06.002
27. Jurga AM, Rojewska E, Piotrowska A, Makuch W, Pilat D, Przewlocka B, et al. Blockade of toll-like receptors (TLR2, TLR4) attenuates pain and potentiates buprenorphine analgesia in a rat neuropathic pain model. Neural Plast. 2016;2016:5238730. [PubMed] [Free full text] DOI: 10.1155/2016/5238730
28. Tanga FY, Nutile-McMenemy N, DeLeo JA. The CNS role of Toll-like Receptor 4 in innate neuroimmunity and painful neuropathy. Proc Natl Acad Sci USA. 2015 Apr 19;102(16):5856–5861. [PubMed] [Free full text] DOI: 10.1073/pnas.0501634102
29. Bettoni I, Comelli F, Rossini C, Granucci F, Giagnoni G, Peri F, et al. Glial TLR4 receptor as new target to treat neuropathic pain: Efficacy of a new receptor antagonist in a model of peripheral nerve injury in mice. Glia. 2008 Sep;56(12):1312-1319. [PubMed] DOI: 10.1002/glia.20699
30. Clark AK, Malcangio M. Fractalkine/CX3CR1 signaling during neuropathic pain. Front Cell Neurosci. 2014 May 7;8:121. [PubMed] [Free full text] DOI: 10.3389/fncel.2014.00121
31. Zhuang ZY, Kawasaki Y, Tan PH, Wen YR, Huang J, Ji RR. Role of the CX3CR1/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following nerve injury-induced cleavage of fractalkine. Brain Behav Immun. 2007 Jul;21(5):642–551. [PubMed] [Free full text] DOI: 10.1016/j.bbi.2006.11.003
32. Staniland AA, Clark AK, Wodarski R, Sasso O, Maione F, D'Acquisto F, et al. Reduced inflammatory and neuropathic pain and decreased spinal microglial response in fractalkine receptor (CX3CR1) knockout mice. J Neurochem. 2010 Aug;114(4):1143–1157. [PubMed] DOI: 10.1111/j.1471-4159.2010.06837.x
33. Hu JH, Yang JP, Liu L, Li CF, Wang LN, Ji FH, et al. Involvement of CX3CR1 in bone cancer pain through the activation of microglia p38 MAPK pathway in the spinal cord. Brain Res. 2012 Jul 17;1465:1–9. [PubMed] DOI: 10.1016/j.brainres.2012.05.020
34. Yang M, Xu W, Wang Y, Jin X, Li Y, Yang Y, et al. CD11b-activated Src signal attenuates neuroinflammatory pain by orchestrating inflammatory and anti-inflammatory cytokines in microglia. Mol Pain. 2018;14:1-12. [PubMed] [Free full text] DOI: 10.1177/1744806918808150
35. Mika J, Osikowicz M, Rojewska E, Korostynski M, Wawrzczak-Bargiela A, Przewlocki R, et al. Differential activation of spinal microglial and astroglial cells in a mouse model of peripheral neuropathic pain. Eur J Pharmacol. 2009 Nov 25;623(1-3):65–72. [PubMed] DOI: 10.1016/j.ejphar.2009.09.030
36. Clark AK, Gentry C, Bradbury EJ, McMahon SB, Malcangio M. Role of spinal microglia in rat models of peripheral nerve injury and inflammation. Eur J Pain. 2007 Feb;11(2):223–230. [PubMed] DOI: 10.1016/j.ejpain.2006.02.003
37. Patro N, Nagayach A, Patro IK. Iba1 expressing microglia in the dorsal root ganglia become activated following peripheral nerve injury in rats. Indian J Exp Biol. 2010 Feb;48(2):110-116. [PubMed]
38. Leinders M, Knaepen L, De Kock M, Sommer C, Hermans E, Deumens R. Up-regulation of spinal microglial Iba-1 expression persists after resolution of neuropathic pain hypersensitivity. Neurosci Lett. 2013 Oct 25;554: 146–150. [PubMed] [Free full text] DOI: 10.1016/j.neulet.2013.08.062
39. Rojewska E, Popiolek-Barczyk K, Jurga AM, Makuch W, Przewlocka B, Mika J. Involvement of pro- and antinociceptive factors in minocycline analgesia in rat neuropathic pain model. J Neuroimmunol. 2014 Dec 15;277(1-2):57–66. [PubMed] [Free full text] DOI: 10.1016/j.jneuroim.2014.09.020
40. Sumitani M, Ueda H, Hozumi J, Inoue R, Kogure T, Yamada Y, et al. Minocycline does not decrease intensity of neuropathic pain, but improves its affective dimension. J Pain Palliat Care Pharmacother. 2016;30(1):1–6. [PubMed] DOI: 10.3109/15360288.2014.1003674
41. Matsumura Y, Yamashita T, Sasaki A, Nakata E, Kohno K, Masuda T, et al. A novel P2X4 receptor-selective antagonist produces anti-allodynic effect in a mouse model of herpetic pain. Sci Rep. 2016 Aug 31;6:32461. [PubMed] [Free full text] DOI: 10.1038/srep32461
42. Yamashita T, Yamamoto S, Zhang J, Kometani M, Tomiyama D, Kohno K, et al. Duloxetine inhibits microglial P2X4 receptor function and alleviates neuropathic pain after peripheral nerve injury. PLoS One. 2016 Oct 21;11(10):e0165189. [PubMed] [Free full text] DOI: 10.1371/journal.pone.0165189
43. Hewitt E, Pitcher T, Rizoska B, Tunblad K, Henderson I, Sahlberg BL, et al. Selective Cathepsin S Inhibition with MIV-247 attenuates mechanical allodynia and enhances the antiallodynic effects of gabapentin and pregabalin in a mouse model of neuropathic pain. J Pharmacol Exp Ther. 2016 Sep;358(3):387–396. [PubMed] [Free full text] DOI: 10.1124/jpet.116.232926
44. Wang X, Zhang Y, Peng Y, Hutchinson MR, Rice KC, Yin H, et al. Pharmacological characterization of the opioid inactive isomers (+)-naltrexone and (+)-naloxone as antagonists of toll-like receptor 4. Br J Pharmacol. 2016 Mar;173(5):856–869. [PubMed] [Free full text] DOI: 10.1111/bph.13394