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Activated Microglia in Nociception

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Microglial cells appear to play a vital role in the initiation of certain neuropathic pain states. In order to initiate neuropathic pain, microglia need to be activated. Microglia activation in the spinal cord involves both hypertrophy as well as hyperplasia, progressing through a hypertrophic morphology, with thickened and retracted processes (observed within the first 24 hours after nerve injury), and an increase in cell number (observed around 2–3 days after nerve injury). There seems to be at least 5 major paths to activate microglia. These 5 pathways will be discussed and are identified by their main signaling mediator and/or receptor which include fractalkine, interferon-gamma, monocyte chemoattractant protein-1, TLR4, and P2X4. Thus, one or more of these mediators/pathways which lead to microglial activation might contribute to neuropathic pain. A greater appreciation of the roles of various mediators/paths which activate microglia might help lead to future novel therapeutic targets in efforts to ameliorate severe symptoms of neuropathic pain.

Key words: microglial cells, glia, C-fiber nociceptors, neuropathic pain, hypertrophy, hyperplasia

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Nociception and glia

Glia account for roughly 70% of the total cell population in the central nervous system. There are 2 types of glial cells: macroglia (oligodendrocytes, astrocytes), and microglia. Spinal glia activation is necessary and sufficient to induce neuropathic pain. Inhibition of spinal glia can effectively attenuate or even block the development of neuropathic pain in many animal models (1-4). It has been demonstrated that peripheral nerve injury can induce spinal microglial/astrocytic activation in several chronic neuropathic pain models (3,5). Activated microglia release a variety of pronociceptive substances that potentiate pain transmission by neurons (6,7).

Microglia appear to have a crucial role in the initiation of processes promoting persistent pain states. Glial activation can be induced by C-fiber nociceptive input from the sciatic nerve (8), and the nociceptive-induced glial activation appears to be crucial in contributing to acute and inflammatory pain in rodent models (9,10). Romero-Sandoval and colleagues (11) suggest that astrocytes play a role in the initiation of acute pain and the maintenance of chronic pain, while microglial activation is important in the early phase or initiation of chronic pain. Furthermore, matrix metal-

loproteinase-9 (MMP-9) might contribute to microglial activation and the "early-phase" of neuropathic pain initiation (likely via interleukin-1 beta cleavage), whereas, MMP-2 might contribute to "late-phase" or maintenance of neuropathic pain in part by astrocyte activation (12).

Peripheral nerve injury induces an early spinal microglial activation that precedes astrocyte activation and implicates astrocytes in the maintenance phase of chronic pain (13). This time course fits the sequential activation of neurons, microglia, and astrocytes, as confirmed by the temporal pattern of activation of mitogen activated protein kinases (MAPK) (14,15).

Activity of peripheral sensory neurons might involve signaling by several molecules. MMP-9 was induced in DRG neurons at the early phase of neuropathic pain development, and MMP-9-deficient mice and mice treated with MMP-9 siRNA showed a reduction of p38 phosphorylation on day one (12). Intrathecal administration of MMP-9 into normal rats resulted in p38 activation with the development of allodynia, and inhibition of p38 prevented MMP-9-induced allodynia. Nerve injury-induced microglial activation markers, including p38 phosphorylation, OX-42 immunostaining, and interleukin-1β (IL-1 β) cleavage, were reduced in MMP-9-deficient mice. Substrates of MMP-9 for microglial activation are unclear, but fractalkine, IL-β and tumor necrosis factor-α (TNF-α) might be potential candidates (TNF-α being the most likely) (16). Microglial p38 activation was suppressed by a CX3CR1 neutralizing antibody (17) and later on microglial p38 activation 3–7 days after nerve injury might involve TLR2, TLR3 and P2Y12R (18-20). Also, MMPs might contribute to neuropathic pain outside of microglia by helping to initiate Schwann cell-mediated myelin basic protein (MBP) degradation and mechanical nociception after nerve damage (21).

There is a theoretic potential that omega-3-polyunsaturated fatty acids (ω-3 PUFAs) might have antinociceptive properties in part by inhibiting microglial release of MMPs. Liuzzi et al (22) have demonstrated inhibitory effects of ω-3 PUFA on microglial MMP-9 expression at the protein level probably resulting from attenuated transcription. Additionally, ω-3 PUFA can down-regulate the activity of the nuclear transcription factors NFkB and AP-1, which are known to be involved in the transcription of the MMP-9 gene (23,24).

Activation of Microglia

In adults, microglia are ubiquitously distributed throughout the central nervous system and have small somata bearing thin and branched processes. Microglia, known as resident macrophages (25,26), make up about 5-10% of the total population of glia (25). Normal microglia have a small soma bearing thin and branched processes and are homogeneously distributed.

Microglia activation in the spinal cord involves both hypertrophy as well as hyperplasia, progressing through a hypertrophic morphology, with thickened and retracted processes (observed within the first 24 hours after nerve injury), and an increase in cell number (observed around 2–3 days after nerve injury). These criteria are immunohistochemical markers for assessing the activation state of microglia in vivo (27) and among them, the change in cell number is the most prominent event. Although the extent of the increase in microglial number is slightly different in different models, peripheral nerve injury increases the number of dorsal horn microglia by 2 to 4 fold (28-32). The enhancement of pain behaviors after nerve injury requires the P2X4 receptor (31) and p38 mitogen-activated protein kinase (MAPK (33, 34), both of which are expressed in activated microglia in the spinal dorsal horn, suggesting that activated microglia may be involved in the pathogenesis of pain hypersensitivity.

Five Paths to Activated Microglia

Although there might be many different ways in which mediators might activate microglia; the following discussion will focus on 5 major pathways which might contribute to microglial activation with respect to nociceptive processes other than MMP-9. These 5 pathways will be identified by their main signaling mediator and/or receptor and include: fractalkine, interferon-gamma, monocyte chemoattractant protein-1, TLR4, and P2X4 (Fig. 1).

Fractalkine

Clark et al (29) report that in preparations of rat spinal cord dorsal horn, with dorsal root-attached from rats with neuropathic pain behavior, preparations, soluble fractalkine (sFKN) contents are increased in the superfusates collected after noxious-like electrical stimulation of ipsilateral primary afferent fibers. The increase in the concentration of sFKN is prevented by morpholinurea-leucine-homophenylalanine-vinyl sulfone-phenyl (LHVS), an irreversible inhibitor of cathepsin S (CatS) whose proteolytic activity is also increased in the superfusates. The source of CatS activity is microglial cells activated by the peripheral nerve injury and secreting the enzyme, as a result of primary afferent

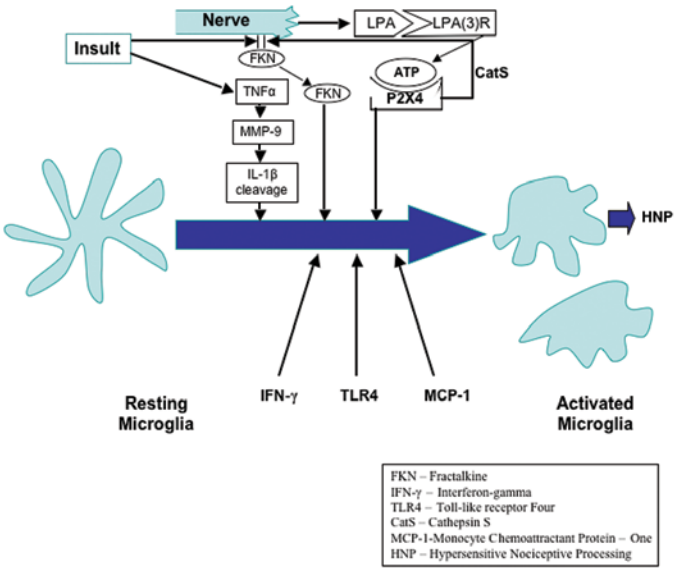

Fig. 1. *Promoters of Microglial Activation*

fiber stimulation. The acute activation of dorsal horn microglia by lipopolysaccharide results in increased CatS activity in the superfusates, followed by increased sFKN contents. Levels of both sFKN and CatS activity in the cerebrospinal fluid increased significantly after peripheral nerve injury, associated with spinal microglial activation. Clark and colleagues (35) found that both FKN immunoreactivity and mRNA become elevated with spinal microglial activation and are confined to dorsal horn neurons. They (35) suggested that under neuropathic conditions, noxious stimulation of primary afferent fibers induces release of CatS from microglia, which liberates FKN from dorsal horn neurons, thereby contributing to the amplification and maintenance of chronic pain.

These data suggest that it is the combination of noxious-like stimulation of primary afferent fibers and nerve injury-induced activation of microglia that results in secretion of CatS, leading to liberation of sFKN in the dorsal horn (35). Noxious-like stimulation of primary afferent fibers alone is insufficient to activate microglial markers or liberate sFKN and CatS in the dorsal horn, and activation of microglia in nerve-injured dorsal horn is not associated with a measurable increase in sFKN and CatS activity, as basal levels of both the chemokine and the protease were comparable to sham slice levels (35). Cathepsin S rapidly induces p38 phosphorylation in spinal microglia after intrathecal injection. This effect is dependent on fractalkine signaling, therefore, fractalkine/CX3CR1 signaling is likely to lead directly to phosphorylation of p38MAPK in microglia (35).

Ceyhan and colleagues (36) demonstrated that CX3CR1 mRNA was overexpressed and protein levels of both CX3CR1 and fractalkine were upregulated in human chronic pancreatitis. Neuro-immunoreactivity for fractalkine and CX3CR1 was strongest in patients suffering from severe pain and pancreatic neuritis. Long-term suffering from chronic pancreatitis appeared to be related to increased neural immunoreactivity of fractalkine (36). Fractalkine and CX3CR1 mRNA overexpressions were associated with enhanced lymphocyte and macrophage infiltration. Advanced fibrosis was associated with increased fractalkine expression, whereas in vitro fractalkine had no significant impact on collagen-1 and alpha-SMA expression in human pancreatic stellate cells (hPSCs). Therefore, Ceyhan and colleagues concluded that pancreatic fractalkine expression appears to be linked to visceral pain and to the recruitment of inflammatory cells into the pancreatic tissue and nerve fibers, with subsequent pancreatic neuritis (36).

Interferon-Gamma

Truda and colleagues found that single intrathecal administration of interferon gamma (IFN-γ) (1,000 U) produced marked and long-lasting tactile allodynia: the paw withdrawal threshold (PWT) to mechanical stimulation applied to the hindpaw which progressively decreased over the first 2 days, peaking between days 2 and 3 ($P < 0.01$), and the decreased PWT persisted for at least 10 days after the administration ($P < 0.05$) (37). Minocycline (40 mg/kg), a glial inhibitor, suppressed the decrease in the PWT at all time points of testing ($P < 0.001$) as well as the activation of microglia in the dorsal horn on day 3 after intrathecal administration of IFN-γ (37).

In wild-type C57BL/6J mice, a marked activation of microglia was observed on the ipsilateral side of the dorsal horn 14 days after nerve injury, as indicated by alterations in Iba1 (ionized calcium-binding adaptor molecule-1) immunofluorescence [a marker of microglial activation], morphology, and number ($P < 0.001$) (37). These alterations in activated microglia were severely impaired in ifngr-/- mice (without IFN- γ R) with nerve injury, and the number of Iba1+ cells was much lower than in wild-type C57BL/6J mice ($P < 0.001$). The number of microglia in the contralateral dorsal horn was similar in these 2 genotypes (37). Behaviorally, wild-type C57BL/6J mice showed a marked decrease in PWT after nerve injury (day one: $P < 0.01$; days 3–14: $P < 0.001$) (37). By contrast, the nerve injury-induced allodynic behavior was strikingly attenuated in Ifngr-/- mice at all time points of testing (days one and 7: $P < 0.05$; days 3 and 5: $P < 0.01$; days 10 and 14: $P < 0.001$) (37).

The loss of IFN-γR did not change either basal mechanical sensitivity or the PWT of the contralateral hindpaw after nerve injury. Nor did IFN-γR deficiency affect motor behaviors in the rotarod test (time on rotarod for wild-type C57BL/6J: 55.4 ± 4.6 sec; for ifngr-/-: 53.2 ±6.8 sec). These findings indicate that IFN-γR-mediated signaling is required for switching spinal microglia to the activated phenotype in the spinal dorsal horn after nerve injury and for producing the subsequent tactile allodynia (37).

In the dorsal horn of wild-type C57BL/6J mice following IFN-γ administration, dorsal horn microglia lacking Lyn tyrosine kinase showed less activated morphology and a smaller increase in number ($P < 0.05$). The loss of Lyn tyrosine kinase also blunted the decrease in PWT following intrathecal administration of IFN-γ ($P < 0.01$). The nerve injury-induced increase in the number of microglial cells in the ipsilateral dorsal horn was lower in lyn-/- mice than in wild-type C57BL/6J mice ($P < 0.001$). These results indicate that Lyn tyrosine kinase is a critical intermediary in the activation of microglia caused by IFN-γ administration and nerve injury (37).

Monocyte Chemoattractant Protein-1

The area in the dorsal horn receiving damaged sensory neurons matches that having markedly activated microglia, therefore, microglial activation requires a signal, or signals, related to "nerve injury." A potential candidate for signaling molecule is monocyte chemoattractant protein-1 (MCP-1 or CCL2), whose expression is markedly increased in dorsal root ganglion (DRG) neurons after nerve injury (38-40). The time-course of MCP-1 upregulation in DRG neurons was similar to that of microglial activation (40). Mice lacking chemotactic cytokine receptor 2 (CCR2), which is a receptor for MCP-1 displayed a reduction of microglia activation caused by nerve injury (41). A spinal MCP-1-neutralizing antibody administered intrathecally inhibited microglial activation (30). Also, intrathecal injection of MCP-1 into normal rats produced an increase in microglial number, as well as allodynia (30).

Peters and Eisenach established a role of the chemokine (C-C motif) ligand 2 (CCL2) to mechanical hypersensitivity after surgical incision in rats (Peters 2010). Twenty-four hours after hind paw incision, rats were intrathecally administered an anti-CCL2 neutralizing antibody (3 and 10 μg) or control immunoglobulin G (10 μg) (Peters 2010). Neutralization of spinal CCL2 acutely reversed mechanical hypersensitivity within 30 min in a dose-dependent manner. A single administration also produced a sustained decrease in mechanical hypersensitivity 48 and 72 hours after incision. Anti-CCL2 antibody reduced microglial activation as measured by the levels of ionized calcium-binding adaptor molecule 1 immunoreactivity and the number of microglia containing phosphorylated p38 mitogen-activated protein kinase 48 hours after incision (42).

TRPV1-positive neurons express the β-chemokine-receptor-2 (CCR2) on their processes in the spinal dorsal horn, and intrathecal administration of its ligand, chemokine CCL2, produced thermal hyperalgesia, which was completely prevented by an antagonist of the CCR2 (43). Up regulation of CCR2 might contribute to sustained excitability of TRPV1-positive nociceptors (44).

Chen et al (45) studied the expression of markers for microglia (ionized calcium-binding adapter molecule 1, Iba1) and astrocytes (glial fibrillary acidic protein, GFAP) in the spinal cord of TRPV1 knockout mice

(KO) vs. wild-type mice (WT) in models of acute (intraplantar capsaicin), inflammatory (adjuvant-induced arthritis, AIA), and neuropathic pain (partial sciatic nerve ligation, PSNL). They reported: naive KO mice had denser immunostaining for both Iba1 and GFAP than naive WT mice; Iba1 immunostaining increased significantly in treated mice, compared to naive mice, 3 days after capsaicin and 7-14 days after AIA or 7-21 days after PSNL, and was significantly greater in WT than in KO mice 3 days after capsaicin, 7-14 days after AIA, and 7 days after PSNL; and GFAP immunostaining increased significantly in treated mice, compared to naive mice, 3 days after capsaicin and 7-21 days after AIA or PSNL, and was significantly greater in WT than in KO mice 14 days after AIA or PSNL. The results suggest that TRPV1 plays a role in the activation of spinal glia in mice with nociceptive, inflammatory, and neuropathic pain.

TLR4

Toll-like receptors (TLRs) are one of the key pattern recognition receptor families that play a critical role in inducing innate and adaptive immune responses in mammals by recognizing a conserved pathogen-associated molecular pattern of invading microbes. At least 13 TLRs have been identified in mammalian species (46,47).

Toll-like receptor 4 (TLR4) is a transmembrane receptor protein with extracellular leucine-rich repeat domains and a cytoplasmic signaling domain. (48,49). In vivo and in vitro studies show that TLR4 is exclusively expressed by microglia (50,51) and leads to NF-κB activation with subsequent induction of proinflammatory cytokines (52,53), thereby contributing to behavioral hypersensitivity. Tanga and colleagues (54) have demonstrated that increased spinal microglial TLR4 activation correlates with the onset of behavioral hypersensitivity in rats after injury to the L5 spinal nerve, even in the absence of exogenous TLR4 ligands such as LPS.

TLR4 mRNA expression is significantly increased in spinal microglia after L5 nerve transaction in rats, a model of neuropathic pain (55). TLR4-knockout and point mutant mice developed less neuropathic pain; these animals showed reduced glial activation and strongly decreased expression of pain related cytokines (55). Also, TLR4 antisense oligonucleotide treatment of neuropathic rats reduced the expression of spinal TLR4; this resulted in a significant attenuation of behavioral hypersensitivity as well as decreased expression of spinal mircoglial markers and diminished proinflammatory cytokines (55).

Bettoni and colleagues (56) showed that after CCI, the TLR4 knockout mice displayed significantly attenuated thermal hyperalgesia and mechanical allodynia compared with their respective wild type controls. They evaluated the efficacy of a potent synthetic TLR4 antagonist (FP-1) administered in mice with painful neuropathy. The repeated treatment of neuropathic mice with FP-1 (5–10 mg/kg, i.p.) evoked a relief of both thermal hyperalgesia and mechanical allodynia, whereas the administration of the highest dose to TLR4 knockout neuropathic mice revealed that in the absence of TLR4 receptor, the compound lost its efficacy (56). As a consequence of TLR4 binding, repeated treatment with FP-1 prevented the activation of the transcription factor NF-kB and TNFα overproduction in the spinal cord (56).

A combination of in vitro TLR4 cell signaling and in vivo behavioral studies of pain modulation suggest that TLR4-enhancement of neuropathic pain and TLR4-suppression of morphine analgesia each likely require HSP90 as a cofactor for the effects observed (57). Hutchinson et al (57) performed a series of studies which explored the involvement of the TLR4 receptor for enhancing certain pain states in vivo. They demonstrated a failure of intrathecally administered lipopolysaccharide (LPS), a classic TLR4 agonist, to produce mechanical allodynia, suggesting that TLR4 might need a co-factor in order to produce functional TLR4 nociceptive signaling. CCI-induced allodynia (a TLR4-dependent form of neuropathic pain) is suppressed by a systemically delivered HSP90 inhibitor (geldanamycin) or by an intrathecally delivered HSP90 inhibitor (17-DMAG) (57). TLR4 signaling in vitro is enhanced by DMSO which releases HSP90 (57). This signaling was blocked by the HSP90 inhibitor, 17-DMAG (56). Furthermore, intrathecal DMSO co-administered with intrathecal LPS induces mechanical allodynia; allodynia induced by intrathecal DMSO co-administered with intrathecal LPS is prevented by blocking HSP90, TLR4, IL-1, or microglial activation (57).

P2X4

Burnstock (58) proposed new roles of nucleotides as neurotransmitters in 1972. Accumulating evidence indicates that nucleotides are released and leaked from nonexcitable cells as well as neurons and are involved in cell-to-cell communication in physiological and pathophysiological conditions (59). In 1995 2 independent groups led by Geoff Burnstock (59) and Alan North of Lewis et al (60), respectively, demonstrated that specific ATP-gated ion channels (P2X2 and P2X3) are localized

on primary sensory neurons and that activation of these channels mediates ATP-evoked neuronal excitability (60,61). These results provided new mechanistic insights into the role of ATP as a specific initiator of acute pain sensations (62-64).

The P2X receptors, of which 7 subtypes (P2X1–P2X7) have been cloned, are a family of ligand-gated cation channels activated by extracellular ATP (65,66) and have important roles in regulating neuronal and glial functions in the nervous system (67,68). Tsuda and colleagues have previously shown that expression of P2X4 receptors (P2X4Rs) in the spinal cord is increased exclusively in microglia after peripheral nerve injury (31). Inhibiting the upregulation of P2X4R prevents tactile allodynia (31). The upregulation of P2X4R in microglia appears to be an important process in contributing to neuropathic pain (3).

When ATP release was measured using a luciferin-luciferase bioluminescence assay, LPA was shown to increase it in an LPA(3) receptor [LPA(3)R] and PLC inhibitor-reversible manner. However, LPA-induced ATP release was also blocked by the Galpha(q/11) AS-ODN, but not by pertussis toxin suggesting that LPA induces the release of ATP from rat primary cultured microglia via the LPA(3) receptor, Galpha(q/11) and PLC (69).

Intrathecal administration of ATP resulted in activated microglia as well as allodynic behavior in rats (69). Coull and colleagues "harvested" resting microglia from control rats that had not been given intrathecal ATP as well as stimulated microglia (ATP-stimulated microglia) from rats that received intrathecal ATP. Intrathecal administration of ATP-stimulated microglia, resulted

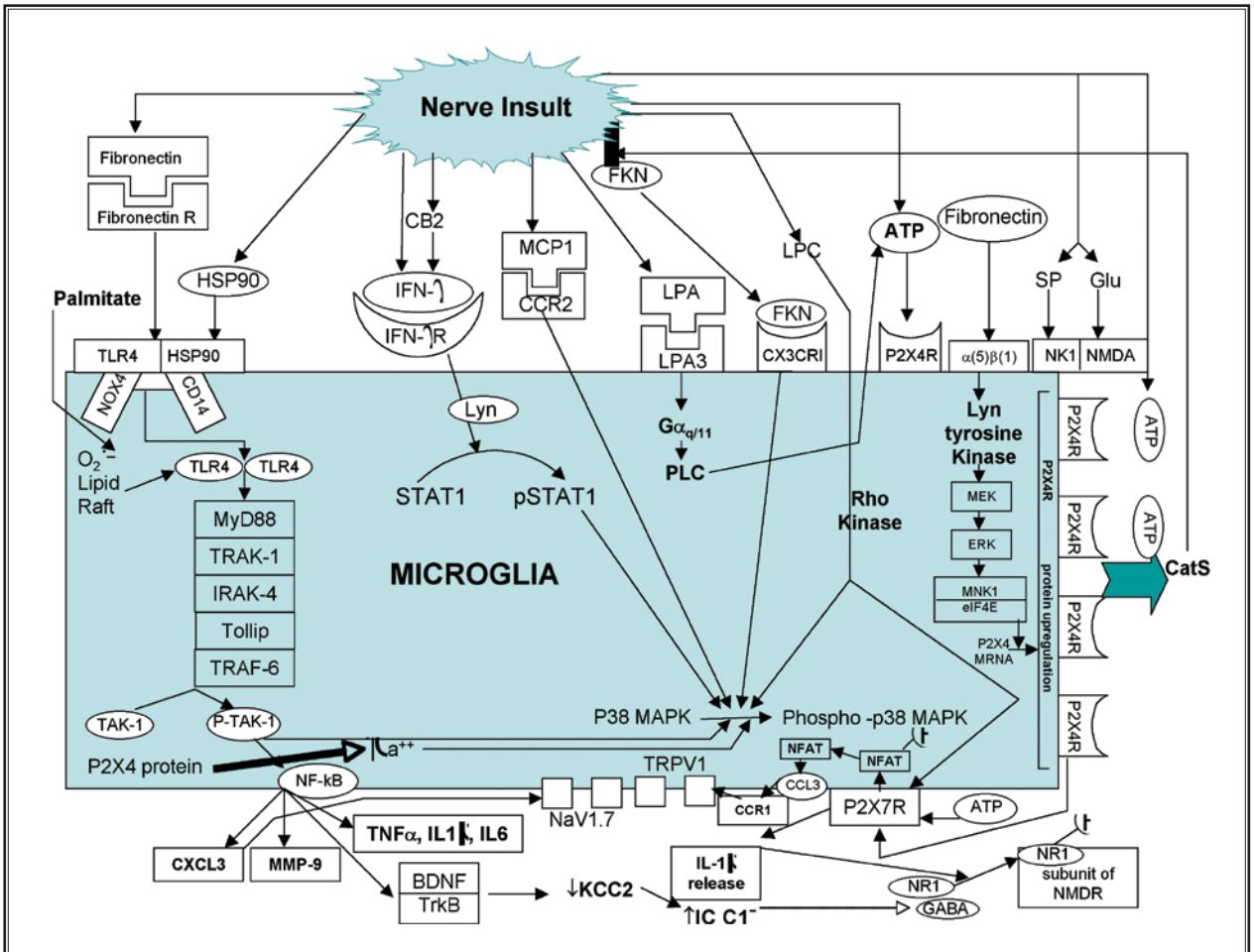

Fig. 2. *Hypothetical schematic of microglial activation and nociception.*

in GABA-evoked deplorization and a depolarizing (e.g. positive) shift of the anion reversal potential (Eanion) in spinal lamina I neurons and allodynic behavior (69). Moreover, TNP-ATP (a P2X receptor blocker) acutely reversed the depolarizing shift in Eanion in lamina I neurons after nerve injury (70). Furthermore, exogenous brain-derived neurotrophic factor (BDNF) or microglial-derived BDNF produced a similar positive depolarizing shift and allodynic behavior that was blocked by bicuculline (a GABAA antagonist) or functional inhibition of BDNF-TrkB signaling (TrkB is the receptor for BDNF) [e.g. anti-TrkB (a function-blocking antibody against the TrkB receptor), or TrkB-Fc (a BDNF-sequestering fusion protein), or BDNF siRNA (Double-stranded short interfering RNA directed against BDNF)] (70). These results suggest that spinal microglia activated by ATP may contribute to neuropathic pain via release of BDNF binding to TrkB receptors with resultant GABAA receptor modulation and a rise in intracellular calcium (Ca++) in spinal lamina I neurons.

Acute pharmacological blockade of P2X4R reverses established tactile allodynia, indicating that tonic activation of P2X4Rs in microglia is necessary for sustaining allodynia. Furthermore, mice lacking P2X4R fail to develop tactile allodynia after nerve injury (31). Moreover, spinal administration of P2X4R-stimulated microglia caused otherwise normal rats to develop allodynia (31). Therefore, P2X4R activation in microglia is not only necessary but is also sufficient to cause tactile allodynia (31).

The level of fibronectin protein was elevated in the dorsal horn 3 to 7 days after nerve injury (71), the time when P2X4R protein levels start to increase (31). Blockade of the fibronectin receptor attenuated nerve injury-induced P2X4R upregulation and allodynia (72). Intrathecal delivery of fibronectin increased P2X4R expression and produced allodynia, a behavior that was not evoked in P2X4R-deficient mice. Cultured microglia, stimulated by fibronectin, showed enhanced levels of P2X4R protein and of ATP-induced Ca2+ influx (71), implying that expression of functional P2X4R in microglia is upregulated by fibronectin stimulation.

Microglia cultured on dishes coated with fibronectin, an extracellular matrix molecule, expressed a higher level of P2X4R protein when compared with those cultured on control dishes. The increase was suppressed by echistatin, a peptide that selectively blocks β1 and β 3-containing integrins, and with a function-blocking antibody of β 1 integrin. In vivo studies show that the upregulation of P2X4Rs in the spinal cord after spinal nerve injury was significantly suppressed by intrathecal administration of echistatin. Tactile allodynia in response to nerve injury and intrathecal administration of ATP- and fibronectin-stimulated microglia was inhibited by echistatin. Intrathecal administration of fibronectin in normal rats increased the level of P2X4R protein in the spinal cord and produced tactile allodynia (72). Nasu-Tada and colleagues (71) found that fibronectin, an EMC molecule, increases P2X4R expression at both the mRNA and protein levels in primary cultured microglia in vitro.

Echistatin preferentially inhibits $\alpha5\beta1$ (73,74), and the expression of $\alpha5$ integrin was detected in microglial cells in vitro (75,76) and in vivo as well (75,77). $\alpha5\beta1$ heterodimers might be the integrins involved in upregulating P2X4Rs by fibronectin, although future studies are needed to determine the involved integrin composition. A fibronectin/integrin-dependent mechanism appears to be important for the P2X4R upregulation in the spinal cord and subsequent neuropathic pain (Fig. 2).

Tsuda et al (78) identified Lyn as a critical signaling molecule for P2X4 upregulation in microglia. Lyn-deficient microglial cells showed a deficit in increased P2X4 expression caused by fibronectin. The level of Lyn expression was increased exclusively in microglia after nerve injury, and Lyn-knockout mice exhibited a striking reduction in upregulation of P2X4 and tactile allodynia after nerve injury. Lyn tyrosine kinase distinctly activated the phosphatidylinositol 3-kinase (PI3K)-Akt and mitogen-activated protein kinase kinase (MAPK kinase, [MEK])-extracellular signal-regulated kinase (ERK) signaling cascades (79,80).

Activated MEK-ERK signaling in microglia exposed to fibronectin enhanced eukaryotic translation initiation factor 4E (eIF4E) phosphorylation status via activated MAPK-interacting protein kinase-1 (MNK1), which might play a role in regulating P2X4 expression at translational levels.

Summary

It appears that the role of microglia in the initiation of neuropathic pain is crucial. There seem to be at least 5 major paths which might lead to microglial activation in certain neuropathic nociceptive states. Pathways are identified by their main signaling mediator and/or receptor and include: fractalkine, interferon-gamma, monocyte chemoattractant protein-1, TLR4, and P2X4. Thus, one or more of the mediators/pathways which lead to microglial activation might contribute to neuropathic pain — alone or in concert. Perhaps some future

cocktail of a fractalkine antagonist, antioxidant, TLR4 inhibitor, interferon-gamma inhibitor, P2X4 inhibitor, and/or TRPV1 antagonists/NK-1 antagonists might be useful to potentially provide analgesia from pain facilitated by microglial activation. A greater appreciation of the roles of various mediators/paths which activate microglia might aid in the discovery of future novel therapeutic targets in efforts to ameliorate severe symptoms of neuropathic pain.

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