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Regulation of pain by neuro-immune interactions between macrophages and nociceptor sensory neurons

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Abstract

Inflammation is the body's protective reaction to injury and infection. Pain is a hallmark of Inflammation and can be either protective or detrimental during acute or chronic phase. Macrophages play a chief role in the pathogenesis of pain and have bilateral communications with nociceptors, the specialized primary sensory neurons that sense pain. Macrophages "talk to" nociceptors by releasing pro-inflammatory mediators (e.g., pro-inflammatory cytokines) that induce pain via direct activation of nociceptors. Macrophages also "listen to" nociceptors, by which nociceptors secrete neuropeptides and chemokines which act on macrophages. Activation of toll-like receptors (TLRs) in nociceptors releases CCL2, activating macrophages and potentiating pathological pain. Emerging evidence also points to a pro-resolution role of macrophages in inflammation and pain. Macrophage GPR37 is activated by neuroprotectin D1, a specialized pro-resolving mediator (SPM) and resolves inflammatory pain via phagocytosis and production of IL-10 that inhibits nociceptors. Macrophage-nociceptor interactions are also mediated by microRNAs and microRNA-containing exosomes in chronic pain. Notably, extracellular microRNAs (e.g., let-7b and miR-711) can directly bind and activate nociceptors. Targeting macrophage-nociceptor interactions will help to control inflammation and pain.

Introduction

Pain is one of the five cardinal symptoms of inflammation [tumor (edema), calor (warmth), dolor (pain), rubor (redness) and fuctio laesa (loss of function)] [1]. The nervous and immune systems are distinct entities with unique structures and functions. During host defense, the nervous system often coordinates with the immune system [2]. While immune cells release mediators to modulate nociceptive sensory neuron (nociceptor) activity,

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nociceptors also release neuropeptides and neurotransmitters to regulate immune cells. Acute pain is an important physiological mechanism to protect animals from potentially hazardous stimuli and environmental dangers. Humans who are born with sensory abnormalities and are unable to detect painful stimuli fail to protect themselves appropriately against harmful stimuli, and this can be life threatening [3]. Persistent or chronic pain, however, is a pathological and maladaptive type of pain with no known benefit to organismal survival. Chronic pain is a clinical epidemic, affecting more than 30% of adults with costs exceeding $600 billion dollars annually in the United States [4].

As a vital protection for organisms, pain is initiated by the activation of primary nociceptors. Nociceptors are unique from other primary sensory neuron populations due to their specialized ability to become activated in response to noxious or potentially damaging stimuli. Peripheral nociceptors represent a heterogenous population which can be defined by their degree of myelination, diameter of soma, electrophysiological properties, and their expression of molecular markers. The cell bodies of primary nociceptors are located in the dorsal root ganglia (DRG) and trigeminal ganglia (TG). These neurons extend afferent projections to peripheral tissues such as skin, muscles, joints, and visceral organs. While conventionally nociceptors were thought to extend free nerve endings to the skin, recently, Abdo *et al.* discovered that specialized cutaneous Schwann cells ensheathe afferent terminals and are able to convey noxious thermal and mechanical sensitivity [5]. At the primary afferent terminals, there are many different molecular sensors expressed on nociceptors such as G-protein coupled receptors (GPCRs), transient receptor potential channels (e.g., TRPA1, TRPV1) sodium channels (e.g., Nav1.7 and Nav1.8), and mechanoreceptors (e.g., Piezo channels) [6,7]. These sensors not only detect physical stimuli such as mechanical injury and noxious temperatures (heat and cold), but can also detect a variety of inflammatory mediators including bradykinin, prostaglandins (e.g., PGE2), ATP, H^+ and noxious inflammatory cytokines and chemokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and CCL2 [6,8] (Figs. 1). In addition, microRNAs may also serve as mediators to pain (Fig. 3), as well as itch, a distinct sensation that triggers scratching [9,10]. After sensing these noxious stimuli, action potentials initiated at the peripheral terminals of nociceptors are carried forward to 2^{nd} order nociceptive neurons in the dorsal horn of the spinal cord or brain stem via their central projections.

Emerging studies have demonstrated that the interaction between the sensory nervous system and the immune system is bidirectional. Peripheral nociceptors can “listen to immune cells” by sensing cytokines released by immune cells. Recent studies have shown that nociceptors also send signals to immune cells to modulate the activity of the immune system [2,11]. Thus, pain is not only an essential sign of inflammatory reactions; it also participates in regulating immunity. After being activated, nociceptors, in turn, release neuropeptides, cytokines, chemokines, and microRNAs, from peripheral terminals or cell bodies to affect resident immune cells such as macrophages, neutrophils, mast cells and dendritic cells [1,12,13].

Polymodal C-fiber nociceptors can be classified into two major subtypes: a neuropeptide-expressing peptidergic population and a non-peptidergic population characterized by its affinity to bind isolectin B4 (IB4). Substance P and calcitonin gene-related peptide (CGRP)

are two of the best-known neuropeptides produced by peptidergic nociceptors and promote pain by neuronal modulation. These neuropeptides are released from nociceptors following intense noxious stimulation and/or activation of TRPA1 and TRPV1 and are critical inducers of neurogenic inflammation [11,14].

Crosstalk between nociceptors and microglia, macrophages in the CNS, has been an area of intense and growing research over the previous two decades. For example, a PubMed inquiry searching the keywords (“microglia” and “neuropathic pain”) yields 1,013 search results from 1994-2019. The contribution of central microglia to chronic pain has been the subject of several comprehensive reviews [15,16]. Peripheral immune cells, however, have received less attention to date. For example, a PubMed query on peripheral macrophages (“macrophage” and “neuropathic pain”) elicits 228 search results over the same time frame, with more than 80% occurring within the last decade. Here, we will discuss this relatively new and hot area of research and highlight the molecular mechanisms by which macrophages contribute to chronic pain initiation, maintenance, and resolution via macrophage-nociceptor crosstalk.

Macrophages induce pain via macrophage-nociceptor interactions

Peripheral macrophages are mononuclear phagocytes which originate from hematopoietic myeloid progenitors in the bone marrow. Macrophages have three major functions: phagocytosis, antigen presentation, and cytokine production. Macrophages also exhibit different functional states [17]. For example, M1-like macrophages exhibit a classically activated phenotype and produce pro-inflammatory cytokines and chemokines to promote pain. In contrast, M2-like macrophages are immunosuppressive cells and secrete anti-inflammatory cytokines and growth factors to promote tissue repair and resolution of pain [18]. It is important to note, however, that macrophages are a heterogeneous population of immune cells, with unique and specialized functions endowed to circulating and distinct tissue-resident populations. Additionally, even among a single population of macrophages, it is increasingly appreciated that these cells exist in a dynamic and non-binary range of functional states that extends beyond the classical M1/M2 nomenclature [19].

Upon activation after tissue injury and infection, macrophages produce various inflammatory mediators, such as inflammatory cytokines, growth factors and lipids that can directly stimulate nociceptors to generate pain. In murine macrophages, substance P increases release of proinflammatory cytokines including TNF-α, IL-1β and IL-6 via activation of ERK-p38 MAPK-mediated NF-κB activation [20] (Fig. 1). Prostaglandin E_2 (PGE_2) is a major inflammatory mediator and induces inflammatory pain via activation of EP1-EP4 receptors on nociceptors. Reactive oxygen species (ROS) can be generated by macrophages to engage TRPA1 in nociceptors [21]. Activation of macrophage angiotensin II type 2 receptor triggers neuropathic pain via oxidative stress and subsequent activation of TRPA1 in nociceptors [22]. Interestingly, TRPA1 activation in Schwann cells was also shown to induce neuropathic pain through interactions with macrophages [23]. Intradermal complement C5a contributes to inflammatory pain via activation of C5aR1 in skin macrophages, and C5a induces thermal hyperalgesia by cooperating with macrophage-NGF and nociceptor-TRPV1 signaling [24].

Macrophages express different TLRs (e.g., TLR4) that regulate the synthesis of proinflammatory cytokines and chemokines. Recently, we demonstrated that macrophage TLR9 signaling promotes chronic pain associated with chemotherapy-induced peripheral neuropathy (CIPN) in male but not female mice [25]. Paclitaxel-induced CIPN is associated with macrophage infiltration and accumulation in DRGs of both sexes. Paclitaxel can directly act on macrophages to induce release of TNF and CXCL1. While hindpaw adoptive transfer of paclitaxel-primed wild-type macrophages is able to induce local mechanical allodynia in both sexes, disrupting TLR9 signaling suppresses pain in male but not female mice. This male-dominant macrophage TLR9-signaling is due to sex-dimorphism in TLR9-triggered release of TNF-α and CXCL1 from macrophages [25]. Future studies are warranted to identify female-dominant macrophage signaling pathways contributing to chronic pain.

Nociceptors also communicate with macrophages via various signal molecules such as neuropeptides (Figs. 1 and 2). In particular, the chemokine CCL2, also known as monocyte chemoattractant protein 1 (MCP1), is a pro-inflammatory chemokine produced by both immune cells (e.g., macrophages) and neurons [26]. In DRG, nerve injury upregulates CCL2 expression in small-diameter neurons, and CCL2 induces peripheral sensitization via activation of CCR2 on TRPV1-expressing nociceptors [27]. In the spinal cord, CCL2 also modulates synaptic plasticity and central sensitization in CCR2-expressing dorsal horn neurons [28]. Abbadie et al. showed that after nerve injury there is infiltration of CCR2-positive monocytes/macrophages to the neuroma and DRG, and furthermore, neuropathic pain is impaired in *Ccr2* knockout mice [29]. Nociceptor-produced CCL2 promotes macrophage infiltration and activation in DRG after nerve injury [30]. Activation of Toll-like receptor (TLR) signaling in nociceptors through myeloid differentiation factor 88 (MyD88) is required for CCL2 synthesis and neuropathic pain [30] as well as chemotherapy-induced neuroinflammation (e.g. innate and adaptive immunity in DRGs) [31] (Fig. 1). Activation of TLR4 in DRG neurons by paclitaxel also causes increased expression of CCL2 by DRG neurons resulting in macrophage infiltration to the DRG [32]. After nerve injury colony stimulating factor 1 (CSF1) is induced in injured DRG neurons and transported to axonal terminals in the spinal cord to induce microgliosis and neuropathic pain [33]. It is likely that CSF1 also contributes to nerve injury-induced macrophage activation in DRG.

Macrophages resolve pain via macrophage-nociceptor interactions

Increasing evidence suggests that macrophages also play an activate role in the resolution of pain by releasing anti-inflammatory mediators such as IL-10 and specialized pro-resolving mediators (SPM) [1]. This action might be mediated by anti-inflammatory M2-like macrophages. Willemen *et al.* demonstrated that G-protein-coupled receptor kinase 2 (GRK2) in LysM(+) myeloid cells control the resolution of inflammatory pain via IL-10 secretion [34]. Loss of GRK2 in myeloid cells was shown to prolong the duration of IL-1β-induced inflammatory hyperalgesia. This deficit can be rescued by adoptive transfer of wild-type bone marrow-derived monocytes. GRK2 deficiency is also associated with decreased production of IL-10 in macrophages [34]. Furthermore, Bang et al. showed that macrophage depletion with clodronate delayed the resolution of heat hyperalgesia and mechanical allodynia in zymosan-induced inflammatory pain [35].

Neuroprotectin D1 (NPD1) is a SPM derived from essential fatty acid - docosahexaenoic acid (DHA)- and displays potent biological actions such as promoting macrophage phagocytosis and restricting neutrophil infiltration. We also demonstrated potent analgesic actions of NPD1 in mouse models of inflammatory pain and neuropathic pain [36,37]. Recently, we identified GPR37 as a receptor of NPD1 to mediate the pro-resolution actions of NPD1 [35]. Bang *et al.* utilized calcium imaging to demonstrate that GPR37 is vital for NPD1 induced intracellular Ca^{2+} increases [35]. We also found that NPD1 could enhance macrophage phagocytosis in vitro via GPR37 by using yeast-derived zymosan. GPR37 upregulates anti-inflammatory cytokines (IL-10, TGF-β) and downregulates proinflammatory cytokines (IL-1β, TNF-α) in zymosan-activated macrophages [35,38]. GPR37-knockout mice showed impaired macrophage phagocytosis of zymosan and apoptotic neutrophils. GPR37 activation favors an M2-like macrophage phenotype and contributes to the resolution of inflammatory pain via increased release of IL-10 and TGF-β [35]. A recent study indicates that GPR37 plays important role in promoting the resolution of inflammation and ischemic stroke in mice [39]. GPR37 is also demonstrated to be expressed in macrophages but not microglia [35]. It is of great interest to test whether GRK2 can be activated by NPD1/GPR37 cascade (Fig. 2).

It is also noteworthy that IL-4-induced M2 macrophages contain and release higher amounts of opioid peptides, such as β-endorphin. Adoptive transfer of M2-macrophages perineurally at the nerve injury site reduced mechanical hypersensitivity, a cardinal feature of neuropathic pain. Interestingly, this anti-neuropathic pain effect of M2-macrophages was compromised by blockade of opioid receptors [40]. Thus, M2-like macrophages may attenuate neuropathic pain via release of opioid peptides, which bind opioid receptors on nociceptors.

Recent evidence suggests that nociceptor activation may also suppress inflammation. CGRP produces anti-inflammatory actions by downregulating cytokine production in myeloid cells. CGRP also modulates the phenotype of TLR4-activated murine macrophages by enhancing expression of the regulatory macrophage markers IL-10 [41] (Fig. 2). In dendritic cells, CGRP inhibits TLR-stimulated production of inflammatory mediators by rapid upregulation of a transcriptional repressor, inducible cAMP early repressor (ICER) [42]. This mechanism may also apply to macrophages. Notably, bacterial pathogens produce pain by directly activating nociceptors but also paradoxically suppress inflammation via release of CGRP from nociceptors [12]. Specific ablation of nociceptors during bacterial infection not only abrogated pain but also increased local inflammation [12]. CGRP release from nociceptors also suppressed neutrophil and γδ T cell responses in lung infections and bacterial pneumonia [43]. It is of interest to investigate the involvement of macrophages in this process.

MicroRNAs regulate macrophage-nociceptor interactions in pain

MicroRNAs (miRNAs) are implicated in neuropathic pain via regulation of gene expression in primary sensory neurons including nociceptors [44]. MiRNAs also regulate macrophage polarization [45]. Cobos Jimenez *et al.* observed that 303 miRNAs were upregulated or downregulated in M1-like and M2-like human macrophages by using miRNA arrays [46]. Exosomes are extracellular vesicles that can be secreted by both immune cells and

nociceptive neurons [47]. Intriguingly, miRNA-containing exomes, released in pain, can communicate between macrophages and nociceptors. Simeoli *et al.* demonstrated that cell bodies of primary sensory neurons have the ability to secrete exosomes containing miR-21 [48]. They also found that exosome secretion from sensory neurons is controlled by TRPV1 activation [48]. Importantly, they demonstrated that exosomes phagocytosis by macrophages has an impact on macrophage phenotype [48]. Overexpression of miR-21 increased pro-inflammatory phenotype of macrophages (M1) and decreased anti-inflammatory phenotype of macrophages (M2) [48]. Both miR-21 antagomir and miR-21 deletion in sensory neurons inhibited macrophage infiltration and neuropathic pain [48]. Willemen *et al.* presented that intrathecal administration of miR-124 can attenuate persistent inflammatory pain [49]. They also demonstrated that miR-124 treatment can decrease ratio of M1/M2 phenotype markers in GRK2-deficient mice and miR-124 can also reduce persistent hyperalgesia in GRK2-deficient mice [49].

Accumulating evidence indicates that extracellular miRNAs can stimulate intracellular communication and exert physiological effects. Lehmann *et al.* demonstrated that let-7b activates TLR7 to induce neuronal apoptosis in cortical and hippocampal neurons [50]. A specific GU-rich motif (GUUGUGU) is the core sequence to stimulate TLR7 and TLR7 mediates cell death through MyD88 [50]. TLR7 is found to express in small-diameter, nociceptive DRG neurons and regulates pruritus and pain [10,51]. Activation of TLR7 by let-7b induced inward currents and elicited action potentials in nociceptors via TLR7 coupling to TRPA1. In this case, MyD88 is not involved in let-7b/TLR7-mediated nociceptor activation [10]. Notably, let-7b is highly expressed in the DRG tissue and activation of nociceptors could significantly increase let-7b release in cultured DRG. Interestingly, they also found that the GU-rich motif (GUUGUGU) was critical for let-7b-induced nociceptor activation in a TLR7- and TRPA1-dependent manner. let-7b was shown to bind TLR7 at the plasma membrane of TRPA1-expressing neurons Since let-7b can also expressed by macrophages [46], it may play an important role during the interaction between nervous and immune systems (Fig. 3).

MiRNAs are not only able to elicit spontaneous pain, they also can evoke itch. Itch is the major symptom of cutaneous T cell lymphoma and Ralfkiaer *et al.* reported five miRNAs were found to be upregulated in the cutaneous lymphoma patients [52]. We found that miR-711 evoked itch without causing pain [9]. By using a combination of electrophysiological recording and calcium imaging, we explain how miR-711 influences TRPA1. We also established a novel cutaneous T cell lymphoma (CTCL) mouse model to demonstrate that miR-711 is an endogenous itch mediator. Finally, the result of computer simulations indicated that GGGACCC which is a core sequence of miR-711 could bind extracellular loop of hTRPA1 [9]. Interestingly, it has been demonstrated that transient receptor potential vanilloid 4 (TRPV4)-expressing macrophages play an active role in chronic itch [53]. Thus, it will be of great interest to investigate whether TRPV4 regulates miRNA release in macrophages.

Conclusion remarks

It is well established that macrophages regulate the pathogenesis of pain through the production of pro-inflammatory and pronociceptive mediators. Recent evidence suggests that macrophages also contribute to the resolution of inflammation and pain by phagocytic clearance of debris and by producing anti-inflammatory and pro-resolving mediators. Compared to acute inflammation which can transmit warning information, sustained inflammation often leads to chronic diseases such as atherosclerosis, chronic pain and even cancer. It is imperative to develop strategies for enhancing phagocytosis and phenotypic switching of macrophages to treat inflammation and chronic pain without significant or with only mild side effects. GPR37 agonists (e.g. NPD1) may be able to selectively target GPR37 in macrophages to treat pathological pain. The sensory nervous system and immune system have an overlapping and synergistic role in recognizing damaging and harmful stimuli. They provide conceptual advancements in our understanding of chronic pain pathogenesis and reveal potential targets for developing therapies for patients with chronic inflammation or chronic pain. Future research on macrophage-nociceptor crosstalk will identify novel mediators and receptors for the control of chronic pain. Other immune cells such as dendritic cells and B cells, may also be important for inflammation-induced pain via neuro-immune interactions. Finally, extracellular miRNAs give us a novel view of interaction between neurons and immune cells and also provide us new targets for treatments of pain or itch-related diseases.

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Highlights

- Macrophages induce pain via pro-inflammatory cytokines that activate nociceptors.
- Nociceptor neurons activate local macrophages via TLR signaling and CCL2 production.
- Macrophages have bilateral interactions with nociceptors via miRNAs.
- Macrophages resolve pain via IL-10 that inhibits nociceptor sensitization.
- Macrophages produce SPM and respond to SPM to resolve inflammation and pain.

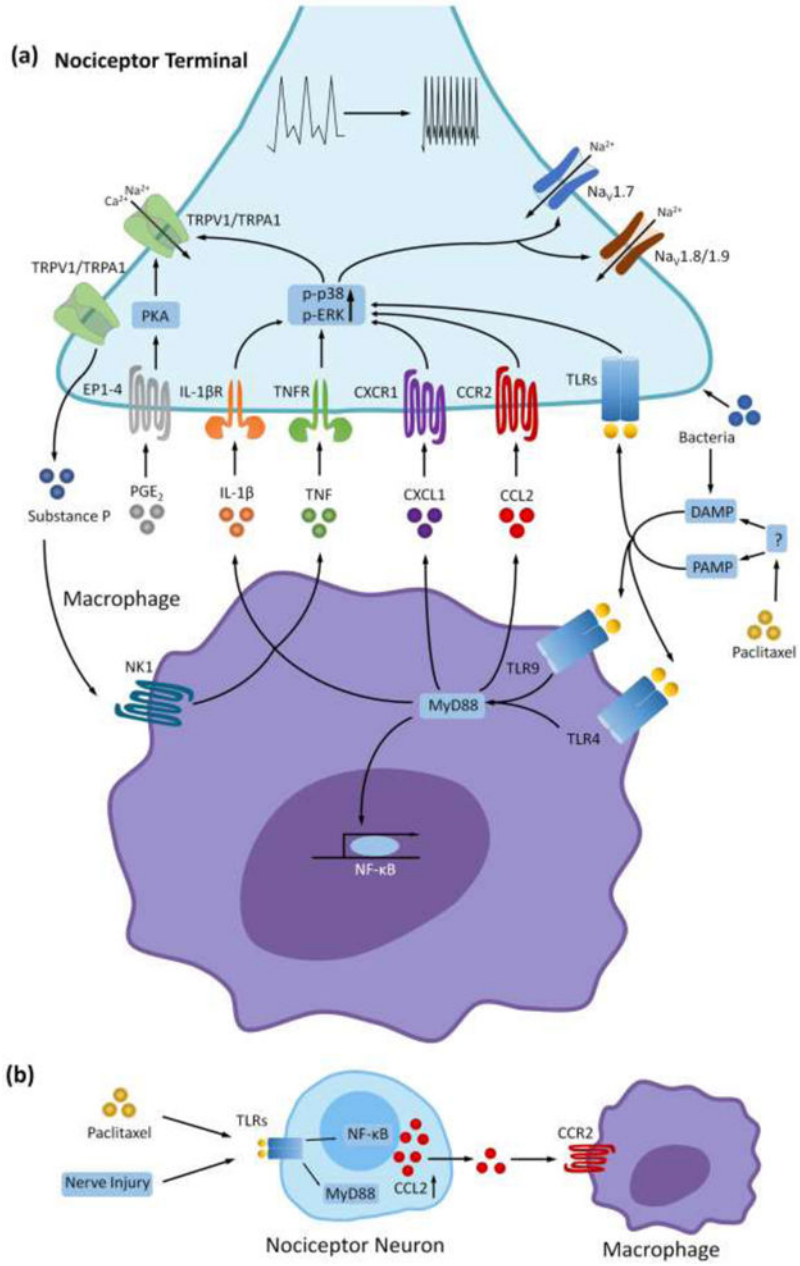

Figure 1. Regulation of pain induction via macrophage-nociceptor interactions.

(a) In macrophages, activation of TLRs (e.g., TLR4 and TLR9) by PAMPs and DAMPs increases the synthesis and release of inflammatory cytokines and chemokines (TNF, IL-1β, CCL2, CXCL1) and lipid mediators (e.g. PGE2) via MyD88 and NF-κB. PAMP and DAMP can be induced by bacteria infection, tissues injury, or chemotherapy (e.g. paclitaxel). These inflammatory mediators act on their respective receptors (e.g. cytokine/chemokine receptors and EP1-EP4 receptors for PGE2) that are expressed on nociceptors, leading to the receptor-mediated signaling transduction through phosphorylation of MAPKs (p-p38 and p-ERK) and activation of protein kinase A (PKA). Upon activation, these kinases then enhance the activities of ion channels (e.g., TRPA1/TRPV1 and voltage-gated sodium channels NaV1.7,

NaV1.8 and NaV1.9) via posttranslational modulations, leading to increased sensitivity and excitability of nociceptors (peripheral sensitization) and increased pain sensitivity. Bacteria is also known to produce pain via specific receptors and ion channels expressed by nociceptors. Furthermore, activation of TRPA1/V1 in nociceptors releases substance P, which binds NK1 receptor on macrophages to release of TNF and IL-1β. In addition, nociceptor neurons express TLRs (e.g. TLR4 and TLR7), and activation of nociceptor TLRs by PAMP and DAMP (e.g. bacteria) can elicit pain. PAMP and DAMP can be indirectly generated by induction of chemotherapy. **(b)** After nerve injury and chemotherapy, nociceptor neurons produce CCL2 via activation of TLRs, MyD88 and NF-κB. CCL2 activates macrophages via CCR2 and induce macrophage infiltration in the DRG and nerve tissues.

Abbreviations: CCL2, Chemokine ligand 2; CCR2, chemokine ligand 2 receptor; DAMP, damage-associated molecular pattern molecules; MAPK, mitogen-activated protein kinase; PAMP, pathogen-associated molecular pattern molecules; TLRs, toll-like receptors

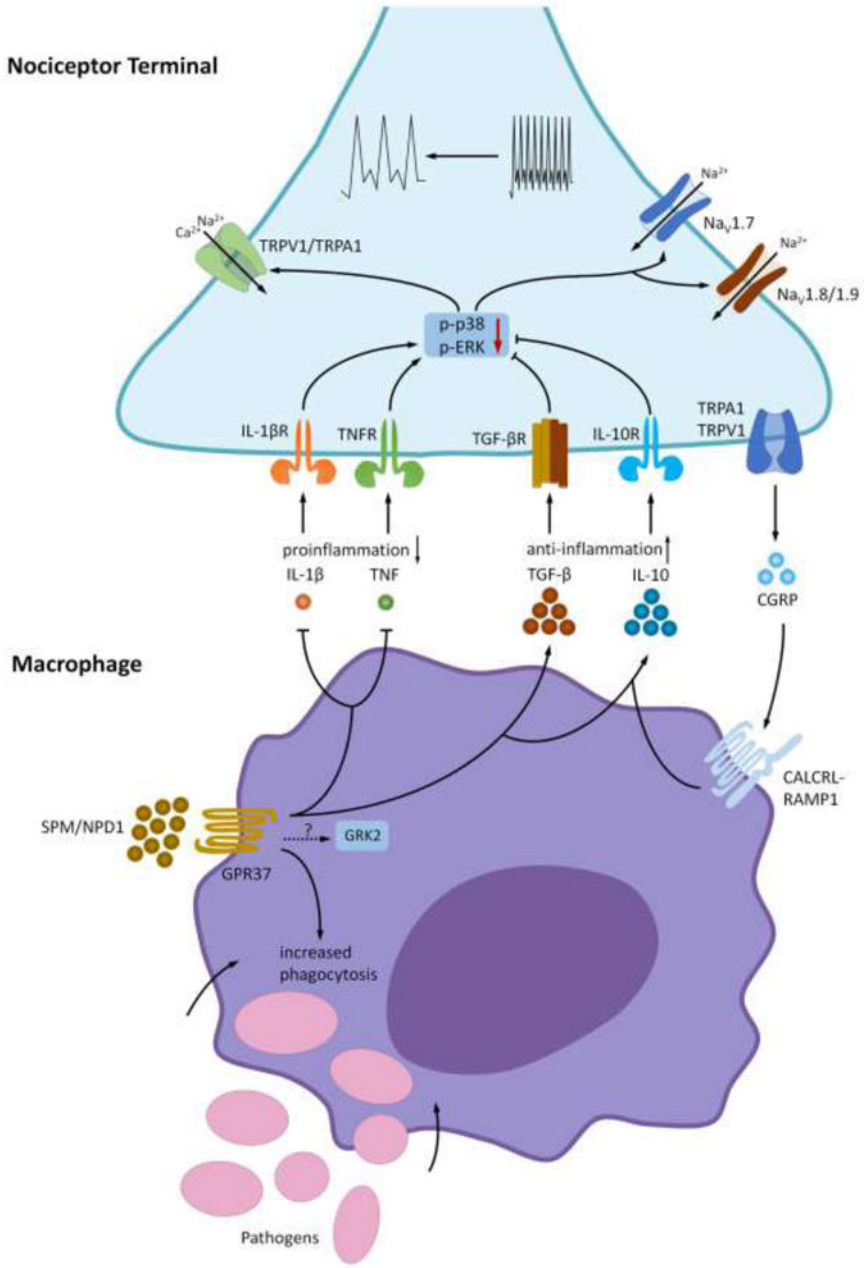

Figure 2. Regulation of pain resolution via macrophage-nociceptor interactions.

(a) Macrophages express GPR37, a newly identified SPM receptor. Activation of macrophage GPR37 by NPD1 promotes phenotypic switch from M1-like macrophages to M2-like macrophages, which contribute to the resolution of inflammation and pain via increased release of IL-10 and TGF-β and decreased release of IL-1β and TNF. IL-10 and TGF-β can act on their respective receptors on nociceptors to inhibit the phosphorylation of MAPKs (p-p38 and pERK) and peripheral sensitization. Activation of GPR37 also induces phagocytosis of pathogens (e.g. zymosman) and apoptotic neutrophils, which is a critical step for the resolution of inflammation and pain. IL-10 production in macrophages is also regulated by GRK2, but the connection between GPR37 and GRK2 is still unknown. In

addition, CGRP released from nociceptor neurons can regulate the anti-inflammatory function via its receptor complex CALCRL-RAMP1 on macrophages to enhance the expression of IL-10.

(b)

Abbreviations: CALCRL, calcitonin receptor-like receptor; NPD1, neuroprotectin D1; RAMP1, receptor activity-modifying protein 1; SPM, specialized pro-resolving mediator

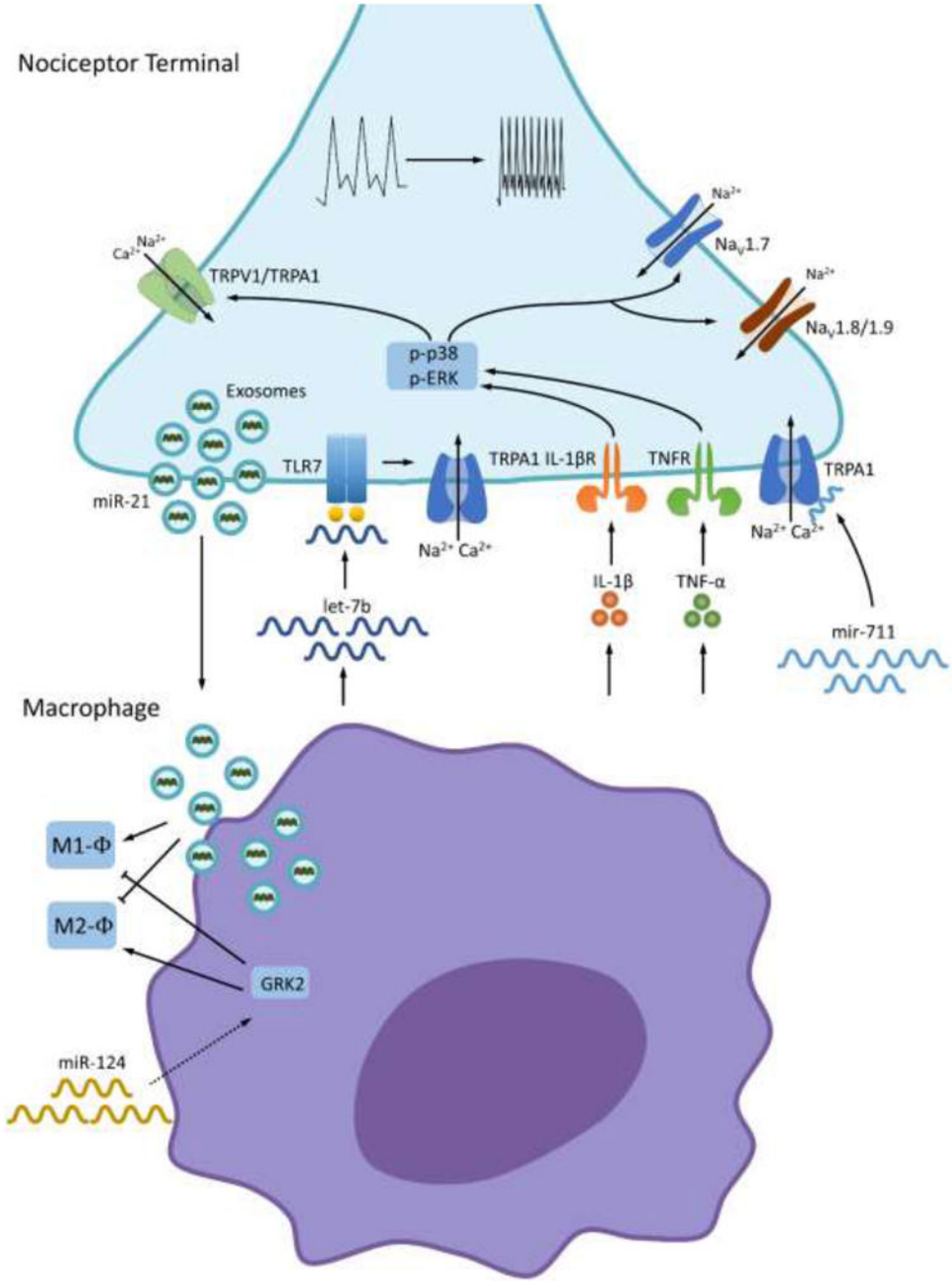

Figure 3. MicroRNA regulation of macrophage-nociceptor interactions.

Nociceptor neurons express miR-21, and miR-21-containing exosomes are secreted following nociceptor activation. Upon secretion, miR-21-containing exosomes are taken up by macrophages. miR-21 enhances neuropathic pain by promoting M1 phenotype and inhibiting M2 phenotype of macrophages (ϕ). In contrast, miR-124 promotes M2 phenotype but inhibits M2 phenotype of macrophages, and this process is mediated by GRK2. Macrophages also produce miRNA let-7b. The secreted let-7b binds with TLR7 via specific motif GUUGUGU to activate nociceptor via TLR7-TRPA1 coupling. Let-7b is also secreted from nociceptors in an activity-dependent manner. In addition, miR-711 is produced by

lymphoma cells and can directly bind and activate TRPA1 in sensory neurons to elicit pruritus.