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·Review·

Emerging role of Toll-like receptors in the control of pain and itch

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Abstract: Toll-like receptors (TLRs) are germline-encoded pattern-recognition receptors that initiate innate immune responses by recognizing molecular structures shared by a wide range of pathogens, known as pathogen-associated molecular patterns (PAMPs). After tissue injury or cellular stress, TLRs also detect endogenous ligands known as danger-associated molecular patterns (DAMPs). TLRs are expressed in both non-neuronal and neuronal cell types in the central nervous system (CNS) and contribute to both infectious and non-infectious disorders in the CNS. Following tissue insult and nerve injury, TLRs (such as TLR2, TLR3, and TLR4) induce the activation of microglia and astrocytes and the production of the proinflammatory cytokines in the spinal cord, leading to the development and maintenance of inflammatory pain and neuropathic pain. In particular, primary sensory neurons, such as nociceptors, express TLRs (e.g., TLR4 and TLR7) to sense exogenous PAMPs and endogenous DAMPs released after tissue injury and cellular stress. These neuronal TLRs are new players in the processing of pain and itch by increasing the excitability of primary sensory neurons. Given the prevalence of chronic pain and itch and the suffering of affected people, insights into TLR signaling in the nervous system will open a new avenue for the management of clinical pain and itch.

Keywords: astrocytes; microglia; Toll-like receptor; pain; itch; danger-associated molecular patterns; pathogen-associated molecular patterns

1 Introduction

Toll-like receptors (TLRs) are the first and best-characterized germ line-encoded pattern-recognition receptors that initiate innate immune responses via recognition of the molecular motifs of pathogens, known as pathogen-associated molecular patterns (PAMPs)[1]. Engagement of TLRs initiates intracellular signaling pathways, leading to the synthesis and secretion of inflammatory cytokines and chemokines, typically by immune cells. TLR-induced innate immune responses are also a prerequisite for the generation of most adaptive immune responses[2]. Therefore, TLRs represent the first line of host defense against pathogens and play a pivotal role in both innate and adaptive immunity[1].

The first TLR family member identified was *Drosophila* Toll, which is essential not only for the antifungal innate immune response but also for dorsoventral patterning during embryonic development[3,4]. Mammalian homologs of Toll were later identified[5]. TLRs are evolutionarily conserved type I transmembrane proteins and comprise an ectodomain, which mediates the recognition of PAMPs and is characterized by leucine-rich repeats, a transmembrane region, and cytosolic Toll-interleukin-1 (IL-1) receptor (TIR)

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domains that activate the downstream signaling pathways[6]. To date, 10 (TLRs1–10) have been identified in humans and 12 functional TLRs (TLRs1–9; TLRs11–13) in mice[6] (Table 1). Based on their subcellular localization, TLRs are divided into surface-expressing (TLRs1, 2, 4, 5, 6 and 10) and intracellular-expressing TLRs (TLRs3, 7/8 and 9) (Table 1). However, some TLRs (e.g., TLR3 and TLR7) are localized both on membrane and in intracellular compartments, such as endosomes and the endoplasmic reticulum, depending on cell type and conditions[1]. While most types of TLRs form homodimers between themselves, some TLRs also form noncovalent dimers, such as TLR1/TLR2 and TLR2/TLR6 heterodimers[7,8] (Fig. 1).

Each type of TLR detects distinct PAMPs derived from microorganisms, such as viruses, bacteria, mycobacteria, fungi, and parasites. For example, TLR1, TLR2, and TLR6 detect lipoproteins[9,10]; TLR3 and TLR7/8 sense double-stranded and single-stranded RNAs, respectively[11-14]; TLR4 responds to lipopolysaccharide (LPS)[15,16]; TLR5 detects flagellin[17]; TLR9 senses CpG DNA[18,19]; and TLR11 senses profilin-like protein[20] (Table 1). Importantly, TLRs can also recognize endogenous ligands[21-29] (Table 1), known as danger-associated molecular patterns (DAMPs) and induce sterile inflammatory responses in many pathological processes, which are known to release DAMPs as a consequence of cell necrosis and tissue remodeling[30]. Thus, the innate immune system is not only activated by TLR recognition of PAMPs but also by TLR recognition of DAMPs released after cell stress and injury.

Most TLRs (but not TLR3) signal through the adaptor protein myeloid differentiation primary response protein 88 (MyD88) (Fig. 1). TLR2 and TLR4 also signal through the adaptor protein TIRAP[31] (Table 1). After recognition of PAMPs by TLRs, MyD88 recruits the IL-1 receptor-associated kinases (IRAKs). IRAK activation results in an interaction with tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6) and recruitment of additional proteins, leading to the phosphorylation of the inhibitor

Table 1. Toll-like receptor (TLR) family members and their subcellular distributions, ligands, and adaptor proteins

TLRs	Localization	Exogenous ligands	Endogenous ligands	Adaptors
TLR1/TLR2	Cell surface	Triacyl lipoproteins, Pam3Cys	HSP (60, 70), HMGB1, urate crystals	MyD88, TIRAP
TLR2/TLR6	Cell surface	Diacyl lipoproteins, PGN, lipoproteins, LTA, zymosan	ω-(2-carboxyethyl)pyrrole	MyD88, TIRAP
TLR3	Intracellular & cell surface	dsRNA, PolyI:C	mRNA, Stathmin	TRIF
TLR4	Cell surface	LPS, Lipid A derivatives	HSP (22, 60, 70, 72), HMGB1, fibronection, Defensin 2, oxLDL, Tenascin C	MyD88, TIRAP, TRIF, TRAM
TLR5	Cell surface	Flagellin	Unknown	MyD88
TLR7	Intracellular & cell surface	ssRNA, imidazoquinoline, loxoribine, bropirimine	Self RNA, siRNA	MyD88
TLR8	Intracellular	ssRNA, imidazoquinoline	Self RNA, siRNA	MyD88
TLR9	Intracellular	Unmethylated CpG DNA, CpG-ODNs	Self DNA, HMGB1	MyD88
TLR11	Cell surface	Uropathogenic bacteria, profilin-like molecules	Unknown	MyD88

CpG-ODNs, CpG-containing oligodeoxynucleotides; dsRNA, double-stranded RNA; HMGB1, high-mobility group box-1; HSP, heat-shock protein; LPS, lipopolysaccharide; LTA, lipoteichoic acid; oxLDL, oxidized low-density lipoprotein; PGN: peptidoglycan; polyI:C, polyinosinic-polycytidylic acid; siRNA, small interfering RNA; ssRNA, single-stranded RNA; TIRAP, TIR domain-containing adaptor protein; TRAM, TRIF-related adaptor molecule; TRIF, TIR domain-containing adaptor protein inducing interferon-β.

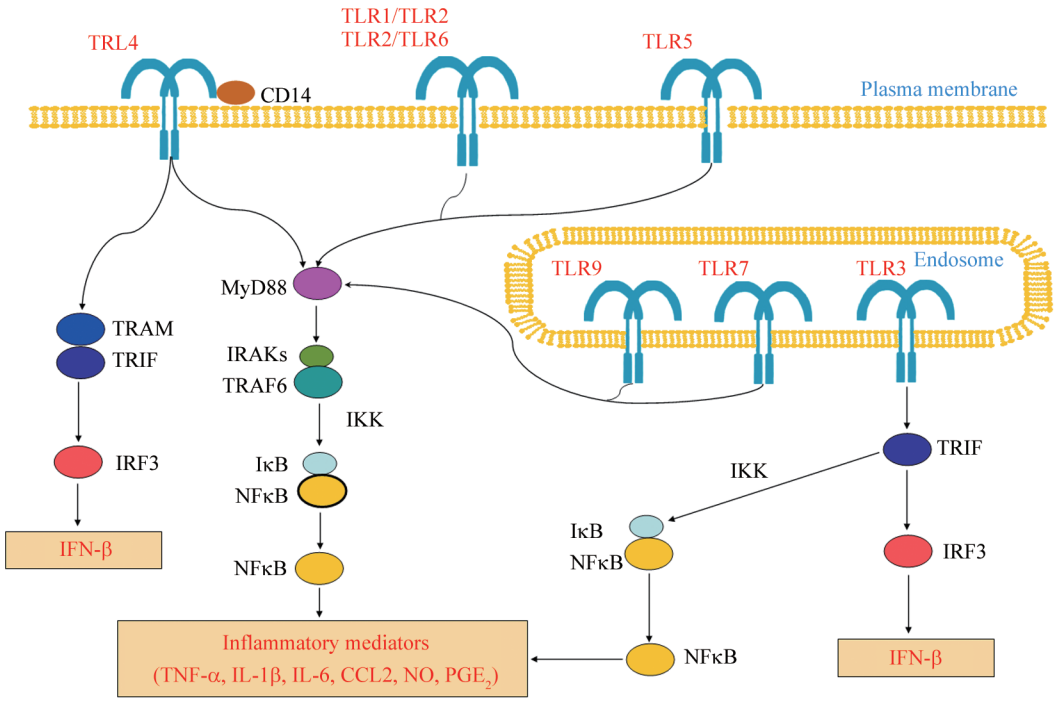

Fig. 1. Schematic of intracellular signaling of Toll-like receptors (TLRs) in mammalian cells. TLR1/TLR2, TLR2/TLR6, TLR4 and TLR5 are expressed on the cell surface for sensing extracellular ligands, whereas TLR3, TLR7 and TLR9 are localized in endosomes for recognizing nucleic acids. All TLRs, except TLR3, recruit the adaptor myeloid differentiation primary response protein 88 (MyD88). TLR1, TLR2, TLR4 and TLR6 recruit the additional adaptor TIR domain-containing adaptor protein (TIRAP), which links the TIR domain with MyD88. MyD88 recruits the interleukin-1 receptor (IL-IR)-associated kinases (IRAKs), which leads to NF-κB inhibitor (IκB) phosphorylation, resulting in activation and nuclear translocation of the key transcriptional factor NF-κB that initiates the transcription of numerous proinflammatory mediators. TLR3 activation initiates the TIR domain-containing adaptor protein inducing IFNβ (TRIF)-dependent pathway. In addition, TLR4 activation can also signal via TRIF-dependent pathways via the adaptor TRIF-related adaptor molecule (TRAM). TRIF interacts with TNFR-associated factor 6 (TRAF6) to activate interferon regulatory factor 3 (IRF3) and initiate the production of type I interferon (IFN). Alternatively, TRIF can also activate the NF-κB and mitogen-activated protein kinase (MAPK) pathways for late-phase induction of proinflammatory mediators. CCL2: chemokine (C-C motif) ligand 2; IKK, IκB kinase; PGE_2, prostaglandin E2; TNF-α, tumor necrosis factor α.

of NFκB (IκB) kinase complex. Phosphorylation of IκB causes the degradation of IκB, allowing the translocation of NFκB to the nucleus and subsequent gene transcription. Simultaneously, TRAF6 also activates the mitogen-activated protein kinase (MAPK) signaling pathways, such as the extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), leading to gene transcription and protein synthesis. Of note, activation of MAPK pathways also causes a rapid release (within 15 min) of inflammatory mediators such as chemokines[32]. Activation of TLR signaling cascades produces a wide array of proinflammatory mediators, including cytokines and chemokines, such as TNF-α, IL-1β, IL-6, IL-12, IL-8 and MIP2, as well as reactive oxygen/nitrogen intermediates such as NO[33] (Fig. 1).

TLRs 3 and 4 also utilize the TIR domain-containing adaptor protein inducing IFNβ (TRIF)-dependent pathway, which culminates in the activation of NFκB and interferon regulatory factor 3 (IRF3)[34] (Fig. 1). TRIF also recruits TRAF6 for NFκB activation similar to the MyD88-dependent pathway. In addition, TRIF recruits a signaling complex which leads to the phosphorylation of IRF3 and its nuclear translocation. Activation of IRF3 leads to the transcription of interferon-β (IFN-β), an anti-inflammatory mediator (Fig. 1). Balanced production of inflammatory cytokines and type I interferon might have a role in controlling tumor growth and autoimmune diseases. The negative regulation of TLR-induced responses is also critical for suppressing inflammation and deleterious immune

responses. TLR activation results in elimination of invading pathogens via recruitment of neutrophils and activation of macrophages, as well as IFN-stimulated gene expression[1]. Moreover, activation of TLR signaling also leads to the maturation of dendritic cells, which is critical for the induction of adaptive immune responses[31].

TLRs are also expressed by many cell types in the central nervous system (CNS) and peripheral nervous system, both non-neuronal cells (e.g. microglia, astrocytes, oligodendrocytes, and Schwann cells) and neurons[35-37]. Activation of TLRs is known to produce various inflammatory mediators including cytokines (e.g. TNF-α), chemokines [e.g. monocyte chemotactic protein-1 (MCP-1)], and enzymes (e.g. cyclooxygenase-2 and matrix metalloproteinase 9), as well as other inflammatory mediators (e.g. prostaglandins)[37-40]. Thus, TLRs play important roles in the pathogenesis of many CNS disorders, including infectious diseases such as CNS viral infection[41] and non-infectious disorders, such as stroke[42], Alzheimer's disease[43], and multiple sclerosis[44].

Increasing evidence indicates that TLRs and their associated signaling components contribute to pain hypersensitivity, and blockade of TLR signaling has been shown to reduce pathological pain[40,45-49]. Spinal application of TLR agonists, such as the TLR4 agonist LPS or the TLR3 agonist polyinosinic-polycytidylic acid (PolyI:C), is sufficient to induce pain-like behaviors in rodents[49-52]. Conversely, blockade of TLR signaling by different strategies attenuates pain in animal models. For example, intrathecal injection of the TLR4 antagonist, LPS of *Rhodobacter sphaeroides*, attenuates arthritic pain[53]. Spinal targeting of TLR4 with specific antisense oligonucleotides[47] or specific siRNA reduces neuropathic[54] and bone cancer pain[55]. Intrathecal injection of green tea-derived epigallocatechin gallate reduces neuropathic pain through inhibition of TLR4 signaling in rats[56]. Intrathecal injection of ketamine also depresses neuropathic pain, at least in part, by inhibiting the TLR3-induced p38 MAPK pathway in microglia[52]. In addition, application of a TLR9 antagonist blocks tumor-induced thermal pain hypersensitivity[57]. Although TLR activation in immune cells must play an important role in pain and itch, this review focuses on the role of TLRs in glial cells (microglia and astrocytes) and primary sensory neurons in processing pain and itch.

2 TLR signaling in spinal cord microglia and astrocytes for chronic pain

Although the molecular and cellular mechanisms underlying the development and maintenance of chronic pain remain unclear, it is generally believed that chronic pain results from neural plasticity including peripheral sensitization[58-60] (in primary sensory neurons) and central sensitization[39,61-65] (in spinal cord and other CNS neurons). Although pain is processed in neural networks, the interactions between neurons and glial cells (e.g. microglia and astrocytes) are also critical for the initiation and maintenance of chronic pain[66]. Increasing evidence suggests that in chronic pain conditions, glial cells are strongly activated in the dorsal root ganglia (DRGs)[67], the spinal cord[68-74], and the brain stem[75]. Activation of glial cells contributes to the pathogenesis of chronic pain via neuron-glial interactions[71,75-78].

Microglial cells are resident innate immune cells originating from primitive myeloid precursors and comprise approximately 10% of the total cells within the CNS[79]. Spinal cord microglia are strongly activated after nerve injury, surgical incision, or chronic opioid exposure[80,81]. Activated microglia not only exhibit increased expression of the microglial markers CD11b and Iba1, but also display elevated phosphorylation of p38 MAPK (Fig. 2A). Activation of p38 in spinal microglia results in increased synthesis and release of the proinflammatory cytokines, such as TNF-α, IL-1β, and brain-derived neurotrophic factor (BDNF)[52,69,82-84]. These microglial mediators powerfully regulate synaptic transmission by enhancing excitatory and suppressing inhibitory synaptic transmission in spinal cord neurons[85-87]. TNF-α and BDNF are also of great importance to the induction of spinal long-term potentiation[83,88-90].

Microglia express most of the TLR family members identified to date. Microglia constitutively express TLR2 and TLR3[91,92] and respond to their agonists for the produc-

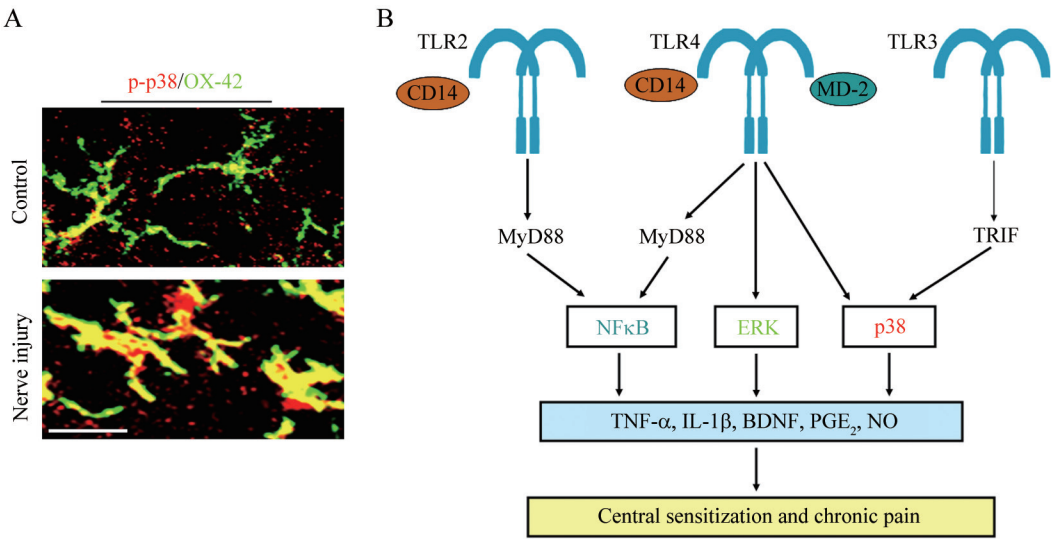

Fig. 2. Toll-like receptor (TLR) signaling in spinal cord microglia for chronic pain sensitization. A: Double staining of phosphorylated p38 (p-p38, red) and the microglial marker CD11b (green) in the superficial spinal cord of rats 3 days after nerve injury (spinal nerve ligation) (unpublished data). Control shows the spinal cord contralateral to the injury. Note that nerve injury induces p38 activation in spinal cord microglia. Scale bar, 20 μm. B: Activation of TLR2, TLR3, and TLR4 in spinal cord microglia results in the activation of the nuclear factor κB (NFκB), extracellular signal-regulated kinase (ERK), and p38 signaling pathways, leading to the production of inflammatory mediators, central sensitization, and chronic pain. BDNF, brain-derived neurotrophic factor; IL-1β, interleukin-1β; MD-2, myeloid differentiation protein-2; MyD88, myeloid differentiation primary response protein 88; PGE_2: prostaglandin E2; TNF-α, tumor necrosis factor α; TRIF, TIR domain-containing adaptor protein inducing IFNβ.

tion of cytokines, including IFN-β, IL-1β and IL-6 (Fig. 2B). TLRs are often up-regulated in microglia following exposure to their own ligands, although TLR3 does not appear to be up-regulated by its ligand polyI:C[92]. TLR9 expressed by microglia responds to CpG DNA to produce proinflammatory mediators and regulate microglial-mediated neurotoxicity[93,94]. Microglia also express TLR7/8, which are highly homologous and important for the expression of microglial proinflammatory mediators and cross-talk with TLR9[92,95]. LPS is one of the best known activators of microglia by activating TLR4[96]. LPS not only induces substantial expression and release of proinflammatory mediators but also causes dramatic morphological changes and proliferation of microglial cells[51,97,98].

Of note, nerve injury-induced microglial activation (e.g. up-regulation of microglial markers) and cytokine expression in the spinal cord are abrogated in TLR4-deficient mice and in wild-type mice treated with TLR4 antisense oligodeoxynucleotides[47]. In addition, TLR2 and TLR3 are required for nerve injury-induced microglial responses in the spinal cord. Antisense knockdown of spinal TLR3 reduces the nerve injury-induced phosphorylation of p38 MAPK in spinal microglia[49,52]. Importantly, nerve injury-induced neuropathic pain is impaired after deletion or inhibition of TLR2, TLR3, or TLR4[47-49,99,100]. In addition to neuropathic pain, spinal TLR4 is important for the microglial reaction after arthritis and the development of chronic arthritic pain[53]. Intrathecal injection of TLR4 ligands is sufficient to induce spinal TNF-α production and persistent mechanical allodynia, a cardinal feature of chronic pain, which is suppressed by minocycline, a non-selective microglial inhibitor[51]. In particular, activation of p38 MAPK in microglia by TLR4 is critical for the release of TNF-α, IL-1β, BDNF, prostaglandin E2 (PGE_2), and NO, leading to pain hypersensitivity[101,102] (Fig. 2).

Astrocytes, derived from neuronal stem cells, are the most abundant glial cell type in the CNS and play various

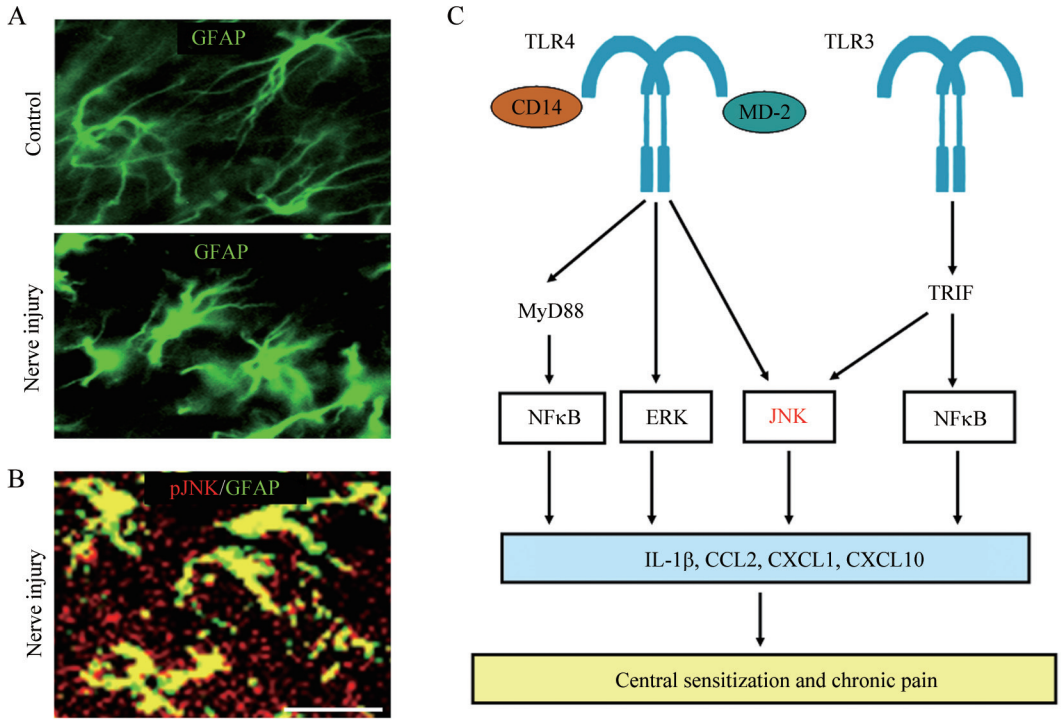

Fig. 3. Toll-like receptor (TLR) signaling in spinal cord astrocytes for chronic pain sensitization. A: Single immunostaining of the astrocyte marker glial fibrillary acidic protein (GFAP) in the superficial spinal cord of control rats and injured rats 7 days after spinal nerve ligation (unpublished data). Note that nerve injury causes hypertrophy of spinal astrocytes. B: Double staining of phosphorylated c-Jun N-terminal kinase (pJNK) and GFAP in the superficial spinal cord of rats 7 days after nerve injury (spinal nerve ligation) (unpublished data). Note that nerve injury induces JNK activation in spinal astrocytes. Scale bar, 20 μm. C: Activation of TLRs (TLR3 and TLR4) in spinal astrocytes results in activation of the nuclear factor κB (NFκB), extracellular signal-regulated kinase (ERK), and JNK signaling pathways, leading to the production of inflammatory mediators, central sensitization, and chronic pain. CCL2, chemokine (C-C motif) ligand 2; CXCL1, CXC-chemokine ligand 1; IL-1β, interleukin-1β; MD-2, myeloid differentiation protein-2; MyD88, myeloid differentiation primary response protein 88; TRIF, TIR domain-containing adaptor protein inducing IFNβ.

functional and structural roles, including formation of the blood-brain barrier, regulation of cerebral blood flow, and participation in the tripartite synapse[103,104]. Astrocytes also play a critical role in chronic pain sensitization[70,105-108]. Following nerve injury, arthritis, or tumor growth, the astrocyte reaction (e.g. up-regulation of GFAP and S100b) is more persistent than the microglial reaction (e.g. up-regulation of CD11b and Iba1) (Fig. 3A), and importantly, astrocyte activation is correlated with chronic pain behaviors[32,108-111]. Nerve injury and inflammation activate ERK and JNK pathways in spinal astrocytes (Fig. 3B), leading to the synthesis and release of inflammatory mediators such as the proinflammatory cytokine IL-1β and the proinflammatory chemokines chemokine (C-C motif) ligand 2 (CCL2, also called MCP-1), CXC-chemokine ligand 1 (CXCL1, KC), and CXCL10 (IP-10)[71,112-114] (Fig. 3B). Of note, IL-1β and CCL2/MCP-1 have been shown to induce central sensitization by increasing the activity of the N-methyl-*D*-aspartate receptor in dorsal horn neurons[32,85,115,116].

Astrocytes express a relatively limited TLR repertoire, in part due to their neuroectodermal origin[117]. Astrocytes express TLR2 and proinflammatory stimuli lead to its augmentation[118]. Astrocytes also express TLR3, and TLR3 activation by polyI:C treatment contributes to proinflammatory phenotypes of human and murine astrocytes via production of proinflammatory mediators such as IL-6[118-120]. Interestingly, astrocytic TLR3 activation also induces the expression of neurotrophic factors and cytokines involved in cellular growth, differentiation and migration[121]. Low,

constitutive expression of TLR4 is detected in astrocytes, and activation of astrocytic TLR4 induces proinflammatory reactions mediated by the NFκB, MAPK and Jak1/Stat1 signaling pathways[118,122]. A recent study has demonstrated that TLR7 activation in astrocytes and its cross-talk with TLR9 signaling regulates the production of proinflammatory mediators[117,118,123]. Astrocytes also express TLR1, TLR5, and TLR6, the functions of which remain, however, unclear[124].

TLRs 2, 3, and 4 regulate spinal astrocyte activation following nerve injury[47-49], although it remains to be investigated whether this activation is secondary to microglial activation by these TLRs. However, stimulation of astrocytic TLR4 with LPS induces strong JNK activation and CCL2 release, which involves TRAF6[125]. Interestingly, TRAF6 is up-regulated in spinal astrocytes after nerve injury, and spinal inhibition of TRAF6 signaling reduces mechanical allodynia[125]. Thus, activation of TLR4 and possibly TLR3 in astrocytes also contributes to chronic pain via activating the JNK/chemokines cascade (Fig. 3B). The detailed mechanisms of TLR signaling in astrocyte activation in chronic pain conditions require further investigation.

3 TLR signaling in primary sensory neurons for pain and itch

Apart from TLR expression in glial cells, increasing evidence has also demonstrated TLR expression in primary sensory neurons, such as DRG and trigeminal ganglion neurons. Expression of TLRs in these primary sensory neurons is involved in pain and itch sensations[40,57,126,127]. It is possible that primary sensory neurons directly detect PAMPs (exogenous TLR ligands) or DAMPs (endogenous TLR ligands) to send warning signals to the brain. Early immunohistochemical analysis revealed that TLR4 and its co-receptor CD14 are expressed in transient receptor potential vanilloid subtype 1 (TRPV1)-expressing trigeminal neurons[126]. Further studies showed that the TLR4 agonist LPS binds to trigeminal neurons, elicits intracellular calcium release and inward currents, and increases TRPV1 activity[128]. In addition, TLR4 colocalizes with calcitonin gene-related peptide (CGRP) in sensory neurons, and LPS enhances the TRPV1-dependent release of CGRP[127]. Recently, it has been shown that activation of TLRs (TLR3, TLR7, and TLR9) in DRG neurons by their respective ligands results in the expression of proinflammatory and pronociceptive mediators such as PGE_2, CGRP, and IL-1β[57]. Of note, mouse DRG neurons require myeloid differentiation protein-1 (MD-1) and CD14 but not MD-2 for TLR4 signaling[129]. The intracellular signaling triggered by neuronal TLR activation needs to be characterized. It is likely that TLR signaling in neurons is distinct from that revealed in immune cells.

Recent evidence also supports a role for TLRs expressed by primary sensory neurons in itch sensation[130]. Itch or pruritus is defined as an unpleasant sensation that elicits the desire or reflex to scratch. Although acute itch serves as a warning and self-protective mechanism[131], chronic itch is a common clinical problem associated with skin diseases[132,133], systemic diseases[134,135], and metabolic disorders[136]. Primary sensory neurons located in the DRG and trigeminal ganglion are responsible for transducing peripheral itch signals to the brain[131,137]. It is well-known that TRPV1-containing C-fibers are required for both histamine-dependent and -independent itch[138,139].

Recently, we showed that functional TLR7 is expressed in small DRG neurons, especially in TRPV1-expressing nociceptors, to mediate itch sensation (Fig. 4A). Immunohistochemistry revealed that TLR7 is highly colocalized with gastrin-releasing peptide (GRP), a neuropeptide known to elicit itch via GRP receptors expressed by superficial dorsal horn neurons[140,141]. Single-cell RT-PCR analysis, conducted selectively in small DRG neurons, also confirmed that TLR7 occurs within the TRPV1 populations (Fig. 4B). Notably, the G protein-coupled receptor MrgprA3, which is known to mediate chloroquine-induced and histamine-independent itch, is completely colocalized with TLR7 (Fig. 4B).

TLR7 recognizes imidazoquinoline derivatives, such as imiquimod and resiquimod (R848), and guanine analogs, such as loxoribine[142]. Intradermal injection of imiquimod, R848, and loxoribine induced itch-indicative

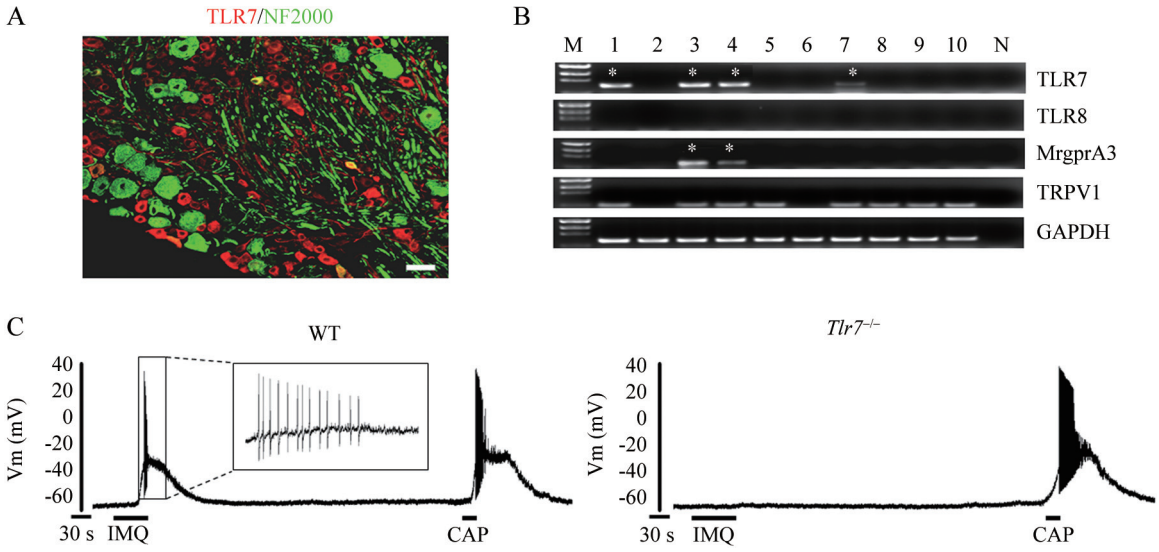

Fig. 4. Dorsal root ganglion (DRG) neurons express functional Toll-like receptor (TLR7). A: Double staining of TLR7 and NF200, a marker for myelinated A-fibers in DRG sections from mice. Note that TLR7 is expressed in NF200-negative and small DRG neurons (unpublished data). Scale bar: 50 μm. B: Single-cell RT-PCR showing colocalization of TLR7 with transient receptor potential vanilloid subtype 1 (TPRV1) and Mas-related G protein-coupled receptor member A3 (MrgprA3) (adapted from Liu *et al.*, Nat Neurosci, 2010[130]). M, molecular weight; N, negative control. Numbers 1–10 indicate the 10 small-sized DRG neurons used for PCR. Asterisks indicate TLR7- and MrgprA3-positive neurons. Single-cell RT-PCR was conducted in small DRG neurons. Note that TLR7-expressing neurons are within the TRPV1-positive population and also include the MrgprA3-positive population. C: Action potentials evoked by imiquimod (IMQ, 500 μmol/L) and capsaicin (CAP, 0.5 μmol/L) in small DRG neurons from wild-type (WT) and *Tlr7* $^{-/-}$ mice (adapted from Liu *et al.*, Nat Neurosci, 2010[130]). Note that imiquimod induces action potentials in wild-type but not in *Tlr7* $^{-/-}$ mice (n = 8 neurons).

scratching behavior in wild-type mice. Importantly, this induced scratching is reduced in *Tlr7*$^{-/-}$ mice, suggesting that these responses are TLR7-dependent[130]. However, imiquimod also elicits TLR7-independent itch[130,143], which may be attributed to its off-target effects, since imiquimod has been shown to act on adenosine receptors or inositol 1, 4, 5-trisphosphate receptors[143-145]. Furthermore, we found that TRPV1-containing C fibers, but not TRPV1 *per se*, are required for imiquimod-elicited itch[130]. Strikingly, application of TLR7 ligands to dissociated DRG neurons elicits very rapid inward currents and action potentials[130] (Fig. 4C). By contrast, these ligands fail to induce inward currents and action potentials in *Tlr7*$^{-/-}$ mice (Fig. 4C). Thus, activation of TLR7 leads to an immediate increase in neuronal excitability. This non-genomic action of TLR7 suggests a possible coupling of TLRs with ion channels in primary sensory neurons that can trigger immediate pain and/or itch sensation.

Compared with wild-type mice, *Tlr7*$^{-/-}$ mice exhibit normal thermal and mechanical pain and unaltered inflammatory and neuropathic pain[130]. Of interest, *Tlr7*$^{-/-}$ mice show a significant reduction in scratching behaviors in response to non-histaminergic pruritogens, including chloroquine, endothlin-1, and SLIGRL-NH2, an agonist of protease-activated receptor 2. Thus, TLR7 is required for histamine-dependent itch but is dispensable for pain sensation.

4 Future perspectives and clinical significance

Despite recent progress and growing interest in understanding the crucial roles of TLR signaling in the regulation of pain and itch, many questions remain unanswered. (1) What are the endogenous ligands for TLRs that are released following cell stress, tissue insult, or nerve injury? And what are the specific contributions of these endogenous TLR ligands to glial and neuronal activation in persistent pain and itch conditions? (2) Is the intracellular signaling of neuronal TLRs distinct from that of immune and glial TLR signaling? (3) What is the role of differ-

ent co-receptors of TLRs that can mediate the interaction between endogenous ligands and TLRs in the processing of pain and itch? (4) What are the molecular mechanisms underlying the excitatory effects of TLR ligands on sensory neurons? (5) In addition to glial cells and sensory neurons, TLRs are expressed in different types of cells in skin tissue, including keratinocytes, Langerhans cells, monocytes/macrophages, dendritic cells, T and B cells, and mast cells[146-148], which are implicated in the pathogenesis of several types of pruritic skin diseases, such as psoriasis, atopic dermatitis, allergic contact dermatitis, and skin infections[147]. Thus, uncovering the precise role of TLR signaling in different types of cells (e.g. neurons, glia, and keratinocytes) for pain and itch sensation requires the generation of conditional knockout mice with specific deletion of TLRs in different cell types. (6) Compared with the well-known roles of TLRs in pain control, much less is known about their roles in the regulation of itch. It remains to be tested whether TLR2, TLR3, and TLR4, the most studied TLR family members that have been implicated in neuropathic pain sensitization, play a role in acute and chronic itch.

Chronic pain, such as tissue injury-induced inflammatory pain and nerve injury-induced neuropathic pain, affects 1.5 billion people worldwide, and current treatments are insufficient[40,149]. Chronic itch is also a common clinical problem associated with skin diseases[132,133], systemic diseases[134,135], and metabolic disorders[136]. Chronic pain and itch substantially reduce the quality of life of affected individuals[150]. Clinically, the current treatments for both chronic pain and chronic itch are far from sufficient[151]. Given their important role in pain and itch, targeting TLRs may offer new treatment for treating debilitating pain and itch-related problems.

Finally, it is important to point out that TLR activation is a double-edged sword, producing both beneficial and detrimental effects[124]. While persistent activation of TLRs could cause chronic inflammation and pathological changes in various diseases, limited activation of TLRs may be beneficial for resolving acute inflammation and restoring homoeostatic balance. Thus, the challenge for developing new therapies is to block the detrimental effects of TLRs, while leaving their beneficial effects intact.

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