Chapter 8
Toll-Like Receptors in Pain and Itch

Amanda S. Andriessen, Elmira Hezarkhani, and Christopher R. Donnelly

Abstract Pattern recognition receptors (PRR) are genetically encoded proteins which recognize a host of highly conserved "danger signals" produced by microbial organisms (pathogen-associated molecular patterns, or PAMPs) or released by damaged host cells (damage-associated molecular patterns, or DAMPs). PAMP or DAMP-mediated activation of PRR-bearing immune cells is a critical step in initiating an immune response. The Toll-like receptors (TLRs) are a small family of proteins with deep evolutionary origins; they are present in both invertebrate and vertebrate species and all TLR members share a common Toll-Interleukin-1 Receptor (TIR) domain. There is considerable variation in the number of TLRs present in different species with Drosophila (9), mice (12), and humans (10) each having a slightly different number which pales in comparison to the number present in purple sea urchins (222). In this chapter, we discuss the basic biology of the TLRs, including their activation, subcellular localization, and downstream signaling. We highlight the critical role that TLRs play in initiating innate and adaptive immune responses and emphasize a discussion of how TLR-mediated proinflammatory signaling is coupled to pain or itch through neuro–immune interactions. We also highlight emerging evidence that suggests TLR signaling in sensory neurons can rapidly modulate neuronal excitability and discuss the physiological consequences of non-canonical TLR signaling in neurons. Overall, this chapter reviews the plethora of evidence which now exists to support the many ways in which TLR signaling can regulate sensory function via neuro–immune interactions.

Keywords Toll-like receptor (TLR) · TLR4 · TLR5 · Toll · Pattern recognition receptor (PRR) · Pathogen-associated molecular pattern (PAMP) · Damage-associated molecular pattern (DAMP) · Immunity · Nociception

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Abbreviations

AP1	Activator Protein-1
BDNF	Brain-Derived Neurotrophic Factor
CIPN	Chemotherapy-Induced Peripheral Neuropathy
CLR	C-type Lectin Receptor
CNS	Central Nervous System
DAMP	Damage-Associated Molecular Pattern
DRG	Dorsal Root Ganglion
ET-1	Endothelin-1
GPCR	G Protein Coupled Receptor
hBD2	Human Beta-Defensin-2
HMGB1	High Mobility Group Box Protein 1
HSP	Heat Shock Protein
IFM	Inflammatory Mediator
IFN-I	Type-I Interferon
IKK	Inhibitor of Nuclear Factor-κB (IκB) Kinase
IL	Interleukin
IL-1R	Interleukin-1 Receptor-Associated Kinase (Biragyn et al. 2002)
ISG	Interferon Stimulated Gene
LPS	Lipopolysaccharide
LTP	Long-Term Potentiation
MAPK	Mitogen-Activated Protein Kinase
MyD88	Myeloid Differentiation Primary Response 88
NF200	Neurofilament 200
NF-κB	Nuclear Factor-κB
NGF	Nerve Growth Factor
NLR	Nucleotide-Binding and Oligomerization Domain (NOD)-Like Receptor
PAMP	Pathogen-Associated Molecular Pattern
PGE_2	Prostaglandin E2
pION	Partial Infraorbital Nerve Ligation
PNS	Peripheral Nervous System
Poly(I:C)	Polyinosinic-Polycytidylic Acid
PRR	Pattern Recognition Receptor
PTX	Paclitaxel
SDH	Spinal Dorsal Horn
sEPSC	Spontaneous Excitatory Postsynaptic Current
SGC	Satellite Glial Cell
sIPSC	Spontaneous Inhibitory Postsynaptic Current
SMOC	Supramolecular Organizing Complex
TBK1	Tank-Binding Kinase-1
TG	Trigeminal Ganglion
TIR	Toll-Interleukin-1 Receptor [Homology Domain]

TIRAP	TIR Domain Containing Adaptor Protein
TLR	Toll-Like receptor
TNF	Tumor Necrosis Factor
TRAF	TNF Receptor Associated Factor (Caterina et al. 2000)
TRIF	TIR-Domain-Containing Adapter-Indued Interferon-Beta
TrkA	Tropomyosin Receptor Kinase A
TRPA1	Transient Receptor Potential Cation Channel Subfamily A Member 1
TRPM8	Transient Receptor Potential Cation Channel Subfamily M Member 8
TRPV1	Transient Receptor Potential Cation Channel Subfamily V Member 1

8.1 A Historical Perspective on Toll-Like Receptors

The overarching purpose of the immune system is to recognize, fight against, and ultimately dispel molecules in our body that we do not recognize and thus could be potentially harmful (Parkin and Cohen 2001). In our conceptual framework of the immune system, we view it as a system divided into two main components: innate immunity and adaptive immunity. Innate immunity encompasses a system designed to provide the first line of defense against invading microorganisms and potential pathogens, conferring rapid, but relatively non-specific protection. In contrast, adaptive immunity, mediated primarily by B and T lymphocytes, takes days or weeks to fully develop, but has the advantage of being specifically directed at a putative danger signal (e.g., a pathogenic microorganism) and also confers immunological memory, wherein the putative danger signal can be "remembered" by adaptive immune cells to enable their rapid activation in subsequent encounters (Parkin and Cohen 2001).

With a few noteworthy examples, our conceptual understanding of how the immune system functions have largely emerged from work performed over the last century. In 1884, Elie Metchnikoff discovered the presence of cells in a species of water flea which engulfed fungal spores, ultimately naming these cells "phagocytes." Through further experiments, his work led to antimicrobial defense, phagocytosis, and rapid microbial detection as key elements of the innate immune system (Modlin 2012), but the molecular mechanisms remained unresolved. An understanding of how the adaptive immune system functions was later to emerge. In 1955, Niels Jerne suggested that an expansive array of soluble antibodies is present in the blood prior to infection, which Frank Burnet further refined in 1957 when he put forth the clonal selection theory. The clonal selection theory suggested that B cells bear a single type of receptor, and its activation, induced by detection of a particular antigen, triggers clonal expansion to generate an expanded lineage of B cells bearing receptors identical to that of the initial parent cell (Burnet 1976). Thus, this theory explained how B cells produce identical antigen-specific clones for humoral antibody-mediated immunity and came to be a foundational concept in our understanding of adaptive immunity (Hodgkin et al. 2007). Thirty years following the work of Burnet, Charles Janeway proposed that innate immune recognition is

coupled with antigen-specific immunity through the use of genetically encoded pattern recognition receptors (PRRs), which recognize exogenous pathogen-associated molecular patterns, or PAMPs (Janeway 2013; Janeway Jr. 1989). Shortly after Janeway's discoveries, Polly Matzinger recognized that inflammation and immunity can also be initiated in the absence of exogenous stimuli, eventually developing the danger model of the immune response which proposed that damaged host cells are likewise capable of releasing signals that can induce immune responses, which eventually came to be known as damage-associated molecular patterns, or DAMPs (Seong and Matzinger 2004).

The understanding of the innate immune system significantly expanded in when Sims et al. cloned the gene for IL-1R1, but the sequence did not point to how Il-1R1 signals as there was a lack of any recognizable pattern in the cytosolic domain (Sims et al. 1988). Three years later, Gay and Keith discovered homology in the amino acid sequence of the IL-1R1 cytosolic domain and that of Toll, a protein in Drosophila melanogaster (Gay and Keith 1991). Toll was already noteworthy for its critical role in Drosophila embryogenesis (Anderson et al. 1985a, b), but the work of Gay and Keith suggested a potentially new function for Toll in inflammation and immunity. Similar to Toll, the drosophila protein, Dorsal, was also known to play an important role in embryonic development in flies, and it was found that the REL homology domain of Dorsal is shared by the mammalian protein nuclear factor kappa B (NF-κB) (Steward 1987). At the time, NF-κB was known to be a transcription factor expressed in lymphocytes (Baeuerle and Henkel 1994), and its drosophila paralog Dif was known to activate transcription of cecropin, an antimicrobial peptide (Ip et al. 1993), suggesting an inducible role for the NF-κB family of transcription factors in immunity. Later, NF-κB was demonstrated to be activated by ligands of Toll and IL-1R1 (Belvin and Anderson 1996; Whitham et al. 1996). Notably, several mammalian proteins were identified which were found to exhibit an even greater degree of homology with Toll than did IL-1R1, sharing the Toll-Interleukin-1 receptor (TIR) domain. Medzhitov and colleagues explored the function of the so-called human Toll (hToll), discovering that hToll activates NF-κB and thus expression of NF-κB-dependent genes (Medzhitov et al. 1997). Shortly thereafter, five additional mammalian Toll homologs were identified and collectively referred to as Toll-like receptors (TLRs). The hToll identified in the previous study is now referred to as TLR4 which will be further explored in following sections.

Today we recognize that TLRs are among the most famous innate immune regulators and are present in all animals from insects to humans, with 10 TLRs conserved in humans and 13 TLRs in mice. Coincident with Charles Janeway's proposed pattern recognition theory, TLRs are the founding members of an expansive family of pattern recognition receptors, yielding an elegant system capable of recognizing and responding to potentially hazardous cues derived from microorganisms (PAMPs) or endogenous host cells (DAMPs). Given that all organisms appear to be at risk of infection, the selective pressure to evolve mechanisms to defend against exogenous and endogenous threats is, and always has been, immense. Thus, PRRs are posited to have early in the history of life, and thus are present in all living things (Janeway 2013; Janeway Jr. 1989).

8.2 TLR Signaling in Immune Cells

Supporting their long evolutionary history and functional significance, all TLRs have a highly conserved structure. They are Type I transmembrane glycoproteins composed of an N-terminal extracellular domain that is leucine-rich, a transmembrane domain, and a C-terminal intracellular TIR domain (Harvey Lodish et al. 2000; Rock et al. 1998). The leucine-rich repeats that make up the ectodomain fold into a solenoid, horseshoe-shaped structure that is inactive until cleaved by endosomal proteases, which are stimulated following TLR-specific ligand interactions. This cleavage of the TLR ectodomain yields functional ability of the TLR to act as a PRR (Botos et al. 2011; Fitzgerald and Kagan 2020; Latz et al. 2007; Ohto et al. 2018; Tanji et al. 2016). All mammalian TLRs examined thus far form direct contact with their ligands through interactions between these ectodomains and their specific ligands. The single transmembrane domain leads to the cytosolic TIR domain, which functions to trigger the downstream signaling of TLRs through interactions with various adaptor proteins.

In accordance with the critical role of TLRs in innate immunity, they are highly expressed by a wide variety of immune cells, with some degree of cell-type specificity (Zuany-Amorim et al. 2002). TLRs also exhibit differential patterns of membrane localization (Fitzgerald and Kagan 2020; Liu et al. 2010a; Randow and Seed 2001). In naïve conditions, TLRs recognizing microbial membrane components such as proteins, lipids, or lipoproteins (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10) are present on the cell surface where they typically encounter their natural agonists (Fig. 8.1). In contrast, nucleic acid sensing TLRs (TLR3, TLR7, TLR8, and TLR9) are typically localized within endosomal compartments, which poises them to detect aberrant intracellular nucleic acids while limiting the degree to which self-derived ligands released by dying cells in the extracellular space promote autoimmune responses (Alexopoulou et al. 2001; Hemmi et al. 2002, 2000) (Fig. 8.1). Cell surface-localized TLRs are typically monomeric, forming homo- and/or heterodimers with other monomers upon ligand binding (Jin et al. 2007; Jin and Lee 2008; Park et al. 2009), whereas endosomal TLRs have been shown to exist as pre-formed homodimers (Ohto et al. 2018; Tanji et al. 2016). Unlike their surface-localized counterparts, the ectodomains of endosomal TLRs must be cleaved by cathepsins to enable dimerization and signal transduction (Ewald et al. 2011; Fukui et al. 2018).

For both cell surface- and endosome-localized TLRs, ligand binding causes conformational changes which bring the cytosolic TIR domains into close proximity. The subsequent detection of dimerized TIR domains by the membrane adaptor proteins TIRAP and TRAM leads to the assembly of an oligomerized scaffold of cytosolic proteins known as supramolecular organizing centers (SMOCs). TIRAP prompts the assembly of the myddosome, which is characterized by the presence of myeloid differentiation primary response 88 (MyD88) oligomers, interleukin-1 receptor-associated kinases (IRAKs), and TNF receptor associated factor-6 (TRAF6), leading to downstream signaling. Loss of MyD88 or other myddosome

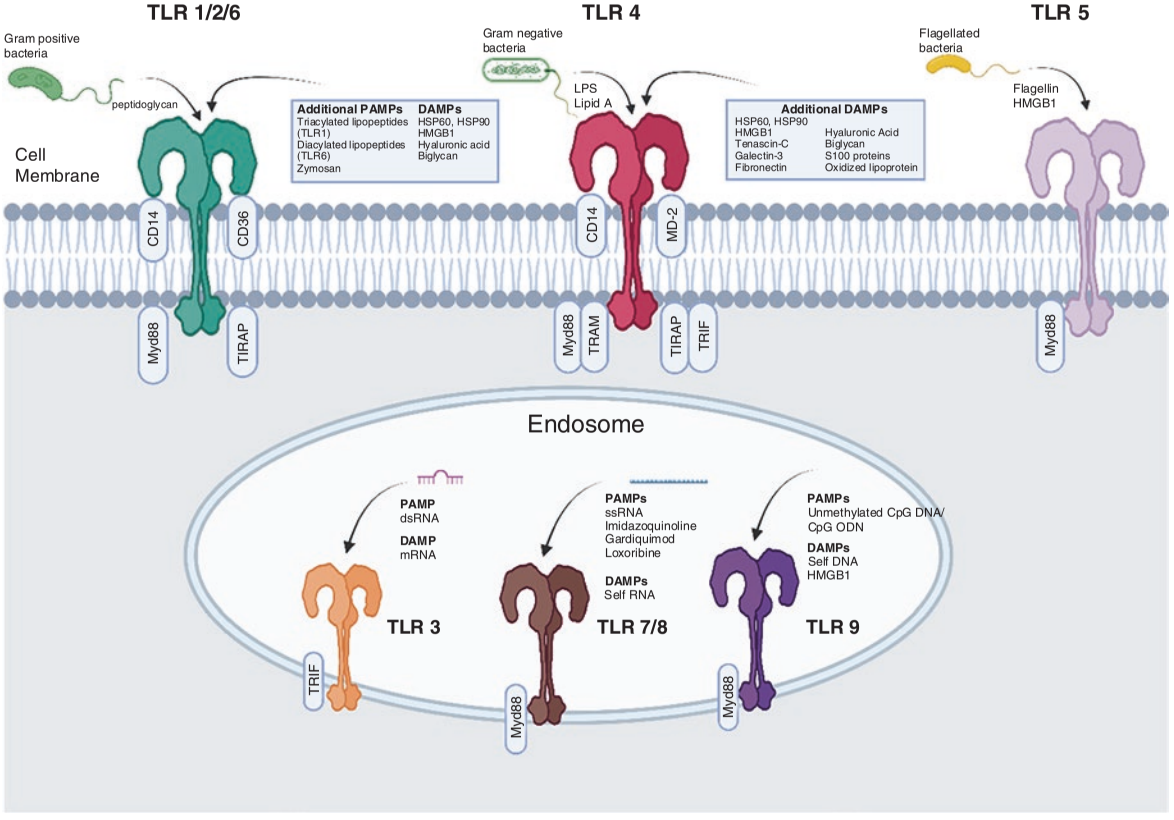

Fig. 8.1 Canonical ligands for Toll-like receptors. TLR 1, 2, 4, 5, and 6 are known as cell surface receptors shown by their placement on the cell membrane. TLRs 3, 7, 8, and 9 are referred to as endosomal receptors as represented by their location within a cellular endosome. It is important to note that while several PAMPs and DAMPs are shown for each TLR, the figure is not comprehensive. PAMPs for TLR 1, 2, and 6 consist of peptidoglycan as shown above, in addition to triacylated lipopeptides (with TLR1), diacylated lipopeptides (with TLR6), and zymosan. DAMPS for these three TLRs consists of HSP60, HSP90, HMGB1, hyaluronic acid, and biglycan. In addition to LPS, Lipid A also serves as a PAMP for TLR4, while HSP60, HSP90, HMGB1, Tenascin-C, Galectin-3, Fibronectin, Hyaluronic acid, Biglycan, S100 proteins, and oxidized lipoproteins function as DAMPs. HMGB1 and Self RNA are DAMPs for TLR5 and TLR7/8, respectively. In addition to the ligands shown for TLR9, HMGB1 also serves as a DAMP for TLR9 (Lacagnina et al. 2018)

components impairs signaling by all TLRs except TLR3 and TLR4 (Akira and Hoshino 2003), and thus, myddosome-mediated signaling is critical for most TLR-mediated functions. Within the myddosome, TRAF6 activates the kinase, TAK1, which stimulates IKK-mediated NF-kB and MAPK/AP-1-mediated transcriptional responses (Emmerich et al. 2013; Wang et al. 2001), as well as recruitment of tank-binding kinase-1 (TBK1). While activation of the transcription factors NF-kB and AP-1 are responsible for the upregulation of inflammatory mediators, myddosome-dependent TBK1 is critical for induction of rapid metabolic changes such as induction of glycolysis (Everts et al. 2014; Fitzgerald and Kagan 2020; Tan and Kagan 2019).

MyD88-independent signaling mediated by TLR4 requires the membrane protein TRAM, which recognizes the homodimers of TLR4, prompting binding to TIR-domain-containing adapter-inducing interferon-β (TRIF) and subsequent TNF receptor associated factor-3 (TRAF3)-dependent activation of the kinase tank-binding kinase-1 (TBK1). This TRIF-dependent, myddosome-independent pathway has been referred to as the triffosome (Fitzgerald and Kagan 2020). TBK1 phosphorylation drives transcription of type-I interferons (IFN-I) and other interferon stimulated genes (ISG) (Fitzgerald et al. 2003a; McWhirter et al. 2004). Although TBK1 is an element of both the myddosome and triffosome, MyD88 lacks a pLxIS motif present within TRIF, and phosphorylation of this motif enables TRIF to interact with the interferon regulatory factor-3 (IRF3) which is critical for TBK1-mediated IFN-I responses. Thus, IFN-I induction is a feature largely restricted to myddosome-independent TLR signaling mediated by the triffosome. Interestingly, it is worth noting that TRAM does not bind to TLR3, and it remains unclear whether a presently unknown adaptor protein with a function analogous to TRAM is required for MyD88-independent TLR3 signaling (Fitzgerald et al. 2003b; Kawai et al. 2001; Navarro and David 1999; Yamamoto et al. 2003a, b).

In summary, TLR activation by PAMPs or DAMPs in immune cells leads to activation of a downstream signaling cascade that varies depending on the TLR in question, but can be broadly divided into two main categories: (1) Myd88/myddosome-mediated TLR signaling, which is employed by all TLRs except TLR3 and is associated with NF-kB and AP-1-dependent transcriptional induction of inflammatory mediators and TRAF6/TBK1-dependent metabolic changes; and (2) TRIF/triffosome-mediated TLR3 and TLR4 signaling, which activates NF-kB, AP-1, and TBK1, yielding an inflammatory response which also features IFN-I (Fig. 8.2). While initially TLR signaling was studied primarily in innate and adaptive immune cells, a plethora of literature has emerged to suggest that other cell types are also capable of participating in inflammation and immunity via canonical TLR signaling (Karikó et al. 2004; Lafon et al. 2006; McKimmie and Fazakerley 2005).

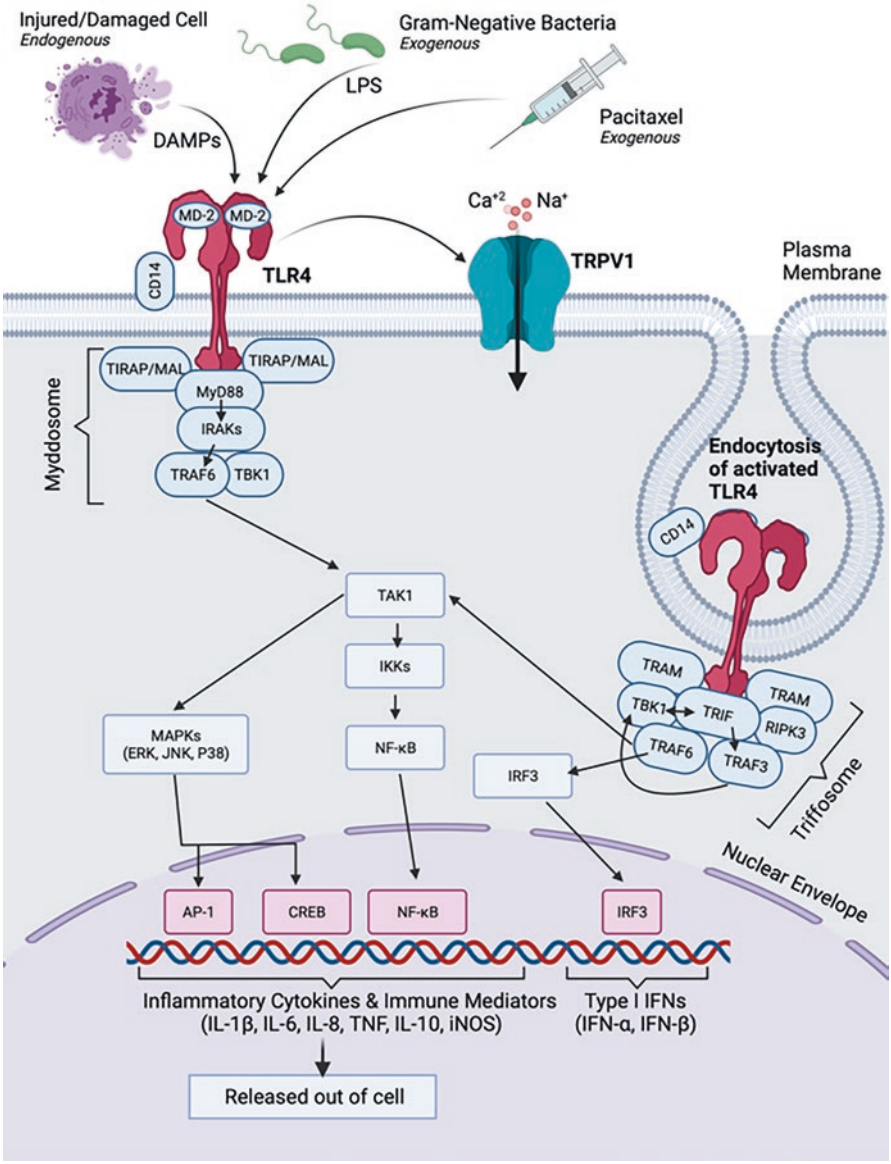

Fig. 8.2 TLR4 signaling in-depth. TLR4 is the most significant and well-known TLR when it comes to pain. This figure displays the downstream effectors of TLR4 activation from DAMPs, LPS, and PTX. There are two SMOCs for TLR4: the myddosome (which forms around most activated TLRs except TLR3) and the MyD88-independent Triffosome (which only forms around TLR4 and TLR3). Activation by a DAMP or PAMP causes formation of a TLR4 dimer on the cell surface, leading to formation of the myddosome, composed of TIRAP, TBK1, TRAF6, and MyD88, which forms around the base of the receptor. This can then activate TNF receptor-associated factor 3 (TRAF3) and downstream kinases such as IRAKs/TRAF6 which come together to form the triffosome. CD14 induces endocytosis upstream of TLR4 signaling and the combined work of CD14 and TLR4 promotes the TRIF signaling in the triffosome (Gay et al. 2014;

8.3 Detecting Danger Is a Shared Responsibility of Immune Cells and Neurons

Hydra, a freshwater metazoan in the Cnidaria phylum, is among the first organisms known to have evolved a nervous system. Notably, Hydra lacks a mesoderm, and thus lacks a defined immune system (Augustin et al. 2017). Given the strong evolutionary selection for organisms to develop host defense machinery, the apparent lack of an immune system is a curious finding and implies other cell types likely contribute to host defense. Interestingly, emerging evidence suggests that the Hydra nervous system plays a direct and active role in host defense, producing antimicrobial peptides and shaping the microbial communities that coat its external body surface (Augustin et al. 2017; Fraune and Bosch 2007). Notably, single cell sequencing analysis of Hydra neurons indicates that they possess elements of TLRs and other pattern recognition receptors, such as NOD-like receptors (NLRs) and C-type lectin receptors (CLRs), and a large proportion of neuronal genes encode antimicrobial peptides (Klimovich et al. 2020). In C. elegans, many components of the innate immune system are missing, and neurons appear to compensate for their loss (Irazoqui et al. 2010a, b; Pujol et al. 2001). For example, nematode sensory neurons mediate avoidance behaviors in response to pathogenic bacteria (Cao and Aballay 2016) and utilizes neuronal GPCR-mediated signaling for the induction or repression of protective immunity in other tissues through many transcriptional mechanisms (Wani et al. 2020). These real-world examples illustrate the highly overlapping role of the nervous system and immune system in protecting organisms from potentially hazardous stimuli and raise questions as to the evolutionary origins of both systems. Appreciating that the very concept of the immune system and nervous system is in some ways an artifact of artificial classification systems, we recognize vertebrates as possessing a well-defined immune system which is separate and distinct in many ways from the nervous system, but an intricate coupling between the two systems exists. Neuronal detection of physical, thermal, and chemical stimuli on a timescale of milliseconds enables rapid behavioral modification to avoid acute threats. In addition, immune cell-derived inflammatory mediators can modulate sensory neuron excitability to draw the attention of an organism to an existing injury or threat leading to protective behavioral responses. In this section we will highlight how TLR signaling in immune cells and PNS- or CNS-resident glial cells contributes to pain through the production of inflammatory mediators. We will also discuss how neuronal TLR signaling is similar and divergent compared to canonical TLR signaling in immune cells.

Fig. 8.2 (continued) Kieser and Kagan 2017). CD14 is an essential component for the subsequent internalization of the TLR and PAMP through endocytosis (Zanoni et al. 2011). Of note, MD2 is the only protein required specifically for TLR4 endocytosis and it has no ability to signal independently of TLR4 (Tan et al. 2015). This process leads to downstream activation of interferon regulatory factors (IRF3), NF-κB, and various MAP kinases and eventually cytokines. Simultaneously TLR4 can directly activate TRPV1 channels to cause cation influx which increases excitability in neurons. TLR4 is an important example of conserved aspects of TLR signaling and non-canonical ion channel coupling (Fitzgerald and Kagan 2020)

8.4 TLR Signaling in Immune Cells Is Indirectly Coupled to Pain via Release of Inflammatory Mediators

The classical view of TLRs signaling in immune cells is coupled to pain through the secretion of inflammatory mediators that sensitize nociceptors. Common among all TLRs, as their canonical immune response function, is to produce inflammatory cytokines which in turn activate resident immune cells, mast cells, and macrophages. Within minutes these immune cells release more proinflammatory mediators and chemokines in addition to other injury response mediators. These activated immune cells and their mediators contribute to peripheral nociceptive sensitization through these factors and interacting directly with nociceptors (Ren and Dubner 2010). All TLRs, except TLR3, use MyD88 as an adaptor in their mechanism which then mainly activates NFκB family members and MAP kinases which then tend to produce a pro-inflammatory response through cytokines like IL-6 and IL-12. TLR3 only uses TRIF which mainly activates IRF family members to induce anti-viral interferon responses (Liu and Ding 2016; Liu et al. 2017; Takeuchi and Akira 2010). Nociceptors express many receptors for these proinflammatory cytokines and chemokines such as IL-1R, TNFR1, TrkA, gp140, and IL-5R which respond to the following TLR-induced upregulated and exocytosed 1L-1β, TNF, NGF, IL-6, and IL-5, respectively. These released IFMs can induce phosphorylation of ligand-gated channels, such as TRPV1 and TRPA1, or modification of voltage-gated sodium channels which leads to increased excitability through changes in neuronal membrane properties, increased firing, and heightened sensitivity to thermal or mechanical stimuli (Pinho-Ribeiro et al. 2017). As an example, one study using a rat L5 spinal nerve ligation model showed that upregulation of TLR3, by way of Poly(I:C) administration, promoted neuropathic pain through promoted expression of inflammatory mediators. Knockdown of TLR3 was able to inhibit SNL-induced microglia autophagy along with relieving mechanical and cold hypersensitivity (Chen and Lu 2017). Additionally, TLR7-mediated recognition of single stranded RNA from both influenza virus and nonviral origins induces production of inflammatory cytokines of the innate immune system, including interferons which have been shown to directly activate sensory neurons in DRG and trigeminal ganglia (Barragán-Iglesias et al. 2020; Diebold Sandra et al. 2004). Painful conditions and disease have been well documented as a result of TLR-mediated proinflammatory processes increase analgesia through direct interaction with nociceptors (Aravalli et al. 2007; Liu and Ding 2016; Nicotra et al. 2012). Thus, TLR activation in immune cells can not only lead to general inflammation but sensitization of nociceptors and pain.

8.5 TLR Signaling in Pain via Neuro-Immune and Neuro-Glia Interactions

While TLRs are classically thought as being expressed by innate and adaptive immune cells, TLRs are also expressed by cell types throughout the peripheral and central nervous systems, including Schwann cells, oligodendrocytes, microglia, astrocytes, and various peripheral and central neuron populations (Bruno et al. 2018). Among peripheral sensory neurons, expression of various TLRs and MyD88 has been demonstrated using multiple experimental methods, including transcriptomic analysis, immunohistochemistry and in situ hybridization, and functional approaches such as electrophysiology and behavioral experiments (Donnelly et al. 2020; Goethals et al. 2010; Liu et al. 2014). However, unlike immune cells, peripheral sensory neurons are electrically excitable cells, bearing ion channels which are coupled, either directly or indirectly, to receptors for inflammatory mediators (IFMs) as well as TLRs. This coupling enables IFMs and TLR ligands to rapidly alter neuronal excitability, leading to behavioral outputs such as pain, analgesia, or itch (Donnelly et al. 2020).

Under homeostatic conditions, DRG-resident satellite glial cells (SGCs) express relatively high levels of *Tlr3* and low levels of *Tlr2* and *Tlr6*. After intraplantar CFA administration, murine *Tlr2* and *Tlr6* are upregulated in SGCs, and agonists of the TLR2/6 heterodimer drive IL-33 production, which directly sensitizes nociceptors (Huang et al. 2020). The contribution of TLR3 in SGCs is unknown. Interestingly, expression of TLR4 in SGCs has been reported to be dependent on neuronal contact, as SGCs isolated from DRG neurons dramatically upregulate Tlr4 (Tse et al. 2014).

Sensory ganglia-infiltrating macrophages are another important contributor to pain pathogenesis after injury, and DRG-infiltrating macrophages have been shown to express several TLRs including TLR2, TLR4, TLR9 (Bruno et al. 2018; Kim et al. 2011; Chen et al. 2020; Luo et al. 2019). Following nerve injury, mice lacking Tlr2 exhibited reduced accumulation of DRG-infiltrating macrophages (Kim et al. 2011; Shi et al. 2011). Given that numerous cell types in the DRG and spinal dorsal horn express TLR4, it has been difficult to identify the specific contribution of TLR4 in macrophages. In rodent models of chemotherapy-induced peripheral neuropathy (CIPN)-associated pain, TLR4 signaling in DRG sensory neurons and spinal microglia are each regarded to contribute to pain pathogenesis (Li et al. 2014). Interestingly, a recent study found that the gut microbiota contributes to CIPN-induced pain, and bone marrow transplant experiments suggested this was through a mechanism involving TLR4 signaling in hematopoietic cells, including macrophages (Shen et al. 2017). TLR9 also contributes to CIPN-induced neuropathic pain, and reportedly does so in male but not female mice (Luo et al. 2019). According to a recent single cell RNA-seq study evaluating several different injury models, a multitude of additional TLRs are induced in neuronal and non-neuronal cell types following injury or insults, including Tlr1, Tlr2, Tlr3, Tlr4, Tlr7, and Tlr9 (Renthal et al. 2020). Thus, it will be interesting to determine how additional TLRs as well as TLR signaling in additional cell types may contribute to acute and chronic pain conditions.

Within the spinal cord, glial cells also express TLRs, and their activation has been shown to increase excitability and sensitization of peripheral sensory neurons through neuro–immune interactions. Microglia, a CNS-resident immune cell which shares many features with peripheral macrophages, are known to express most TLRs, which enables them to detect a variety of PAMPs and DAMPs and initiate protective immunity (Bsibsi et al. 2002; Olson and Miller 2004). Following nerve injury, murine spinal cord glial cells isolated from Tlr2 knockout (KO) mice exhibit reduced expression of TNF, IL-1β, IL-6, and iNOS genes comparison to WT glial cells, and Tlr2 KO mice exhibit reduced mechanical allodynia and thermal hyperalgesia, suggesting glial Tlr2-mediated IFMs are an important contributor to neuronal sensitization and neuropathic pain after injury (Kim et al. 2007). In both rodents and humans, the TLR4 ligand LPS is regarded as one of the best-known activators of microglia, inducing robust production and release of IFMs and causing marked microglial activation and persistent pain (Clark et al. 2010; Saito et al. 2010). Tlr4 KO mice exhibit reduced neuropathic pain behaviors following nerve injury and a concomitant reduction in microglia proliferation and pro-inflammatory cytokine induction in the spinal cord (Tanga et al. 2005). Intrathecal injection of TLR4 antagonists reverses nerve injury-induced mechanical allodynia and thermal hyperalgesia in mice and rats (Bettoni et al. 2008; Nicotra et al. 2012). In microglia, TLR4-mediated activation of p38 MAPK is critical for the release of TNF, IL-1β, BDNF, prostaglandin E2 (PGE2), and nitric oxide, all of which contribute to pain hypersensitivity (Chen et al. 2018; Saito et al. 2010).

Compared to microglia, astrocytes express a more limited repertoire of TLRs, and fewer studies exist which have studied the role of TLR signaling in astrocytes in pain pathogenesis. In rodents, TLR2, TLR3, and TLR4 regulate spinal astrocyte activation following nerve injury (Kim et al. 2007; Tanga et al. 2005) and appear to be the main TLRs expressed in astrocytes. A recent single cell RNA-seq analysis of murine brain astrocytes found that Tlr3 is expressed most abundantly, with more modest levels of Tlr2. This study failed to detect Tlr4, although more sensitive methods have shown low levels of constitutive expression of Tlr4 in astrocytes (Hasel et al. 2021). TLR3 activation by extracellular poly I:C treatment prompts astrocytes to produce pro-inflammatory mediators (Scumpia et al. 2005). LPS stimulation of astrocytes induces proinflammatory cytokine production (Carpentier et al. 2005; Gorina et al. 2011). TLR4 signaling in astrocytes contributes to the pathogenesis of CIPN-associated neuropathic pain in mice and rats (Li et al. 2014).

8.6 Non-canonical TLR Signaling in Sensory Neurons via Coupling to Ion Channels

The transient receptor potential (TRP) channel family is the largest and most widely studied family of noxious stimulus detectors (Patapoutian et al. 2009). This family is composed of 28 structurally similar non-selective ligand-gated cation channels divided into multiple subfamilies. As discussed in previous chapters, TRP channels

are critical for sensing various thermal and cold stimuli, and can be activated by naturally occurring chemical ligands (e.g., capsaicin for TRPV1, menthol for TRPM8, and mustard oil for TRPA1) (Moore et al. 2018). TRPV1 and TRPA1 are well-documented to play a critical roles in neurogenic inflammation, neuronal sensitization, acute and chronic pain, and itch (Bautista et al. 2006; Caterina et al. 2000; Donnelly et al. 2020; Liu et al. 2016, 2010b; Patapoutian et al. 2009). Notably, RNA-Seq studies have demonstrated DRGs expression TLR1, TLR2, TLR3, TLR4, and TLR5 in both mice and non-human primates (Kupari et al. 2021; Usoskin et al. 2015; Yang et al. 2019; Zeisel et al. 2018). With one noteworthy exception which we discuss later (TLR5), most TLRs are selectively enriched in nociceptors. Interestingly, many studies have demonstrated that TLRs are co-expressed with TRPV1 or TRPA1, and TLR activation by PAMPs or DAMPs can rapidly modulate neuronal excitability through functional coupling to TRPV1 or TRPA1 (Barajon et al. 2009; Fitzgerald and Kagan 2020; Lacagnina et al. 2018; Liu et al. 2012a; Materazzi et al. 2012; Park et al. 2014; Qi et al. 2011).

Among TLRs exhibiting expression in sensory neurons, TLR4 is the most widely studied. TLR4 is important for the initiation and establishment of chronic pain as well as other inflammatory pathologies (Bruno et al. 2018; Gao et al. 2017; Mohammad Hosseini et al. 2015; Wu et al. 2010; Zuany-Amorim et al. 2002). While much of this has been attributed to TLR4 signaling in non-neuronal cells, contributing to pain via neuro–immune or neuro–glia interactions, TLR4 is also highly expressed in DRG nociceptors (Bruno et al. 2018; Donnelly et al. 2020; Zuany-Amorim et al. 2002). The prototypical PAMP, lipopolysaccharide (LPS, also known as endotoxin) signals through TLR4 by binding its co-receptor myeloid differentiation protein-2 (MD-2) in the TLR4 binding cavity. TLR4 also responds to various DAMPs, including heat shock proteins, extracellular matrix degradation products, and high-mobility group box-1 (HMGB1) (Fig. 8.3) (Beutler and Rietschel 2003; Botos et al. 2011; Kelly et al. 2006). Acute application of LPS to dissociated trigeminal ganglion (TG) neurons evokes inward currents and increased Ca2+ influx within a timescale of seconds to minutes (Diogenes et al. 2011). Rapid capsaicin-induced pain behaviors are reduced in global TLR4 knockout mice, and capsaicin-induced Ca2+ influx is reduced in Tlr4 deficient DRG neurons (Min et al. 2018). Notably, co-IP experiments suggest that TLR4 and TRPV1 physically interact via the TIR domain of TLR4, which prevents internalization of TRPV1. TLR4 signaling in peripheral sensory neurons was also recently reported to contribute to the early onset of nerve injury-induced neuropathic pain behaviors in female mice, but not male mice, via HMGB1-mediated TLR4 activation in nociceptors (Szabo-Pardi et al. 2021). This study did not explore whether these effects were TRPV1-dependent. Thus, TLR4 signaling in nociceptors contributes to pain, possibly via multiple cellular mechanisms, including direct coupling to TPRV1.

Although the central dogma is that nucleic acid sensing TLRs such as TLR3 and TLR7 are localized to endosomal membranes, recent studies have suggested surface localization can occur in sensory neurons and some immune cell types (Kanno et al. 2015; Liu et al. 2010b; Mielcarska et al. 2021; Park et al. 2014). In DRG neurons, TLR7 was reported to be expressed by small-diameter nociceptors and colocalized

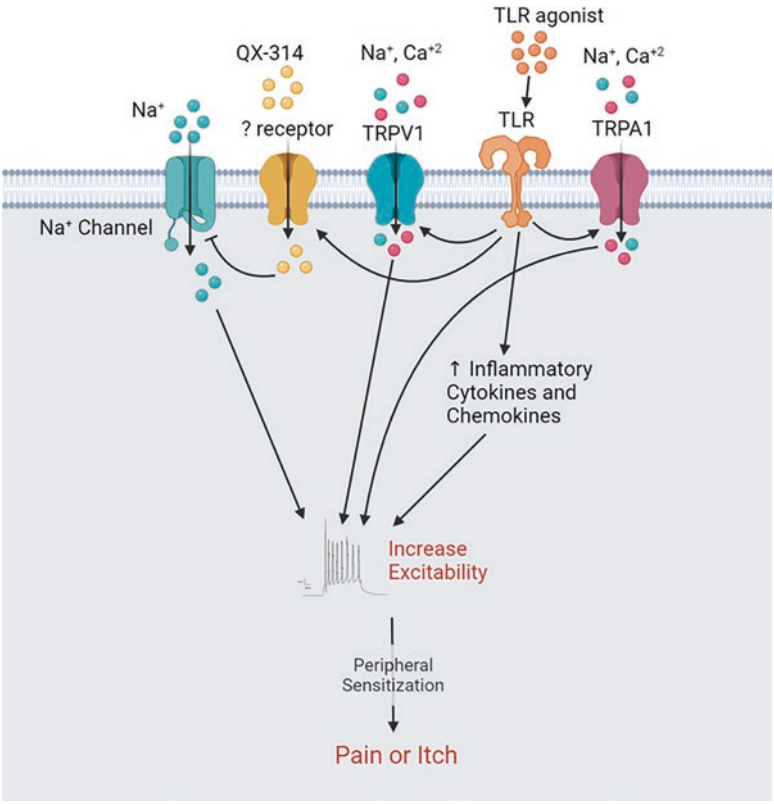

Fig. 8.3 Overview of TLR mechanisms in neurons TLRs are well known to increase inflammatory cytokines and chemokines yet these factors have various effects beyond innate and adaptive immunity. Upon activation of a TLR it can lead to activation of TRPV1, TRPA1, or an unknown receptor of QX-314 in addition to canonical upregulation of pro-inflammatory cytokines and chemokines. The production of IFMs as well as TRPV1 and TRPA1 activation leads to increased neuronal excitability and in turn causes peripheral sensitization of sensory neurons, experienced as pain or itch. The activation of the receptor of QX-314, such as from TLR5 activation, will inhibit sodium ion influx which would otherwise increase the neuronal excitability

on the cell surface with TPRV1 (Liu et al.). Intraplantar administration of the microRNA (miRNA) let-7b elicits rapid pain-related behaviors in mice, and miR-let-7b perfusion rapidly induces inward currents and action potentials in DRG nociceptors. These actions were reported to be dependent on functional coupling between TLR7 and TRPA1, wherein TLR7-mediated detection of let-7b modulates neuronal excitability via TRPA1 (Park et al. 2014). These studies suggest that damaged host cells may release miRNAs, which act as DAMPs, activating TLR7 in nociceptors to rapidly promote pain. Tlr3 is also expressed in TRPV1+ nociceptors (Barajon et al. 2009; Liu et al. 2012a) and appears to be dispensable for acute pain, but important for central sensitization-mediated pain hypersensitivity. The TLR3 agonists Poly(I:C) and dsDNA rapidly induce inward currents and action potentials

in DRG neurons, an effect which is abolished in *Tlr3* KO mice. Recordings from lamina II neurons of the spinal dorsal horn in slice preparations found that Poly(I:C) induced a TLR3-dependent increase in the frequency of spontaneous excitatory post-synaptic currents (sEPSCs), and tetanic stimulation of C-fibers induced long-term potentiation (LTP) of the field potential in WT, but not Tlr3-deficient mice (Liu et al. 2012a). Activation of TLR3 was also shown to upregulate TRPV1 and rapidly enhanced TRPV1-mediated Ca^{2+} flux (Qi et al. 2011). Thus, activation of TLR3 in nociceptors promotes central sensitization through coupling to TRPV1, although the precise mechanism remains unknown.

Historically, TLR8 has been less studied than its colleagues TLR7 and TLR3 since it was believed to be non-functional in mice; however, studies showing its role in cell apoptosis and upregulation in the CNS in response to microbial activation have since brought it more attention (Ma et al. 2007; Olson and Miller 2004). One such recent study demonstrated that while acute itch, pain sensation, and inflammatory pain were not impaired by TLR8-/-, chronic pain in a spinal nerve ligation (SNL) model was significantly reduced in the TLR8 knockout mice. Subsequent imaging showed that not only was TLR8 highly expressed in small-diameter, mainly nonpeptidergic IB4+, neurons, but the intensity of TLR8 was increased post-SNL at all timepoints (3, 20, and 21 days) (Zhang et al. 2018). TLR8 has been shown to play a similar role in the other collection of primary sensory neurons, trigeminal ganglia (TG). In a model of partial infraorbital nerve ligation (pIONL), similar to SNL, TLR8 has enduring increased expression in TG neurons. Genetic deletion of TLR8 with pIONL not only attenuated mechanical allodynia but the expected effects of reduced activation of ERK and p38-MAPK and reduced pro-inflammatory cytokines, for example, TNF-α, Il-1β (Zhao et al. 2021). In both the TG and DRG models of nerve ligation, timing of TLR8 knockdown was an important factor that contributed to the conclusion that TLR8 is required for the maintenance of neuropathic pain. Additionally, TLR8 agonists, VTX-2337, in naïve mice alone is enough to produce mechanical allodynia based on nocifensive behavior (for TG-directed pain) and paw-withdrawal (for DRG-directed pain) (Zhang et al. 2018; Zhao et al. 2021).

8.7 TLR5 and Mechanical Allodynia

TLR5 is unique in that it is one of the few TLRs that recognizes a protein PAMP, bacterial flagellin, which is a critical component of bacterial flagella in both Gram-negative and -positive bacteria (Hayashi et al. 2001). While it is highly expressed in the intestinal mucosal epithelium, immunohistochemistry, in situ hybridization, and RNA-sequencing studies demonstrate that TLR5 is also highly expressed by large-diameter neurofilament-200 (NF-200)-positive DRG sensory neurons (Gewirtz et al. 2001; Xu et al. 2015; Yang et al. 2019). In fact, in situ hybridization showed that nearly all (91%) of Tlr5+ neurons exhibit NF200-positive immunoreactivity, and that the vast majority (78%) of NF200-positive neurons co-express Tlr5+.

NF200 is a marker for large-diameter, low-threshold, myelinated A-fiber neurons which are responsible for mechanical allodynia (Ahn et al. 2012; Campbell et al. 1988; Ohsawa et al. 2013; Ossipov et al. 2002). Application of the TLR5 ligand, flagellin, induces calcium responses in mouse DRG neurons. Additionally, co-application of flagellin with QX-314, a positively charged membrane-impermeable lidocaine derivative, suppresses mechanical allodynia in animal models of chemotherapy-induced peripheral neuropathy (CIPN), sciatic nerve injury, and diabetic neuropathy-induced neuropathic pain. This co-application selectively blocks action potentials by suppressing sodium currents, thereby suppressing Aβ-fiber conduction to attenuate neuropathic pain behaviors in both naive and CIPN mice. However, the effect is lost in *Tlr5* deficient mice, emphasizing that it is TLR5-mediated (Xu et al. 2015). This has since been followed up in a chronic constriction injury (CCI) model of neuropathic pain in rats where activation of TLR5, via flagellin administration, relieved mechanical hyperalgesia and mechanical allodynia for up to 6 h post-injection (Chang et al. 2021). In the CNS, TLR5 is expressed in microglia and has been shown to modulate their activity in response to flagellin or brain injury. However, in alignment with the canonical view of TLRs, microglial activation of TLR5 in the brain appears to exacerbate nerve injury-induced neuroinflammation and neuropathic pain (Ifuku et al. 2020).

8.8 TLR Signaling in Itch Pathogenesis

As discussed in previous chapters, pruriception, or the sensation of itch, serves as an additional sensory mechanism to aid in the detection of potential environmental hazards or threats. Scratching behavior activates reward circuits, which in turn evoke pleasurable feelings along with an increased desire to scratch (Su et al. 2019). There are several distinct subtypes of itch, including: touch-evoked itch (mechanical itch), chemical itch, opioid-induced itch, and chronic itch, a condition in which itching lasts for longer than 6 weeks Isaac M. Chiu (2018). Chronic itch can occur as a consequence of many conditions beyond dermatological diseases, including neoplasms (cutaneous T-cell lymphoma), systemic diseases (e.g., chronic liver disease, end-stage kidney diseases), and metabolic disorders (Chen et al. 2021; Kremer et al. 2020; Kurban et al. 2008). As discussed in previous chapters, the peripheral and central neural circuits responsible for itch is a rapidly developing field that has primarily emerged within the last 15 years (Dong and Dong 2018). In peripheral sensory ganglia, itch-sensing neurons are regarded as a subpopulation of nociceptors, termed pruriceptors, although TRPV1 and TRPA1 are expressed in both populations (Ji 2015). In this section we will review how TLRs contribute to itch pathogenesis via neuro–immune interactions or via sensory neuron-intrinsic mechanisms involving coupling with ion channels.

TLRs are expressed by several cell types within the skin, including keratinocytes and Langerhans cells in the epidermis, and macrophages, dendritic cells, and mast cells within the dermis (Miller 2008). Keratinocytes, the major epidermal cell type,

are frequently the first responders to exogenous pathogens or injury. In keratinocytes obtained from psoriatic skin lesions, expression of several TLRs is elevated leading to proinflammatory cytokine signaling which exacerbates skin inflammation (Cristina Lebre et al. 2003; Lebre et al. 2007). In chronic itch patients with atopic dermatitis, prurigo nodularis, and psoriasis, protein levels of TLR3 are increased in lesional skin compared to non-lesional skin or healthy skin. Human keratinocytes treated with the TLR3 agonist poly(I:C) show increased expression of endothelin-1 (ET-1), which stimulates mouse DRG neurons to release of B-type natriuretic peptide (Szöllősi et al. 2019), a known neuropeptide uniquely involved in pruritus (Shimizu et al. 2014). Notably, ET-1 is also elevated in lesional skin in prurigo nodularis patients (Kido-Nakahara et al. 2014), suggesting this mechanism may be conserved in humans. As mentioned in the previous section, small-diameter DRG neurons, usually considered nociceptors, express TLR3, which contributes to central sensitization after an acute pain stimulus. Itch-sensing sensory neurons, or pruriceptors, are a subpopulation of nociceptors, and TLR3 is expressed within a subset of TRPV1+, GRP+ pruriceptors. Interestingly, intradermal injection of the TLR3 agonist poly(I:C) induces robust scratching behaviors in mice, which are abolished in Tlr3 KO mice. Scratching behaviors induced by intradermal pruritogens were also attenuated in mice lacking Tlr3, and TLR3-deficient mice did not exhibit dry skin-induced scratching behaviors unlike WT controls. This appears to be directly dependent on TLR3 signaling in pruriceptors, as acute perfusion with RNA extracted from skin or poly(I:C) induces inward currents in TLR3+ DRG neurons (Ji 2015).

Similar to TLR3, TLR7 has been demonstrated to play important roles in both pain and itch. TLR7 is expressed in a subpopulation of pruriceptors, only some of which express the GPCR MrgprA3, which is responsible for histamine-independent, chloroquine-dependent itch behaviors. TLR7 agonists induce dose-dependent scratching behaviors in mice, and dose-dependent inward currents in DRG neurons, both of which are abolished in Tlr7 KO mice (Liu et al. 2012b). Interestingly, pruritus is a common side effect of the TLR7 agonist imiquimod when applied topically in human cancer patients (Madan et al. 2010) Similar to TLR3, TLR2 is expressed in mouse DRG and TG neurons, and mice lacking TLR2 exhibit attenuated acute and chronic itch behaviors, as well as reduced formalin-induced inflammatory pain. Activation of TLR1/2 heterodimers using the agonist Pam3CSK4 evoked both pain and itch, whereas activation of TLR2/6 heterodimers using lipoteichoic acid and zymosan produced only pain (Wang et al. 2020). These effects were attributed to direct activation of DRG neurons, as Pam3CSK4 and zymosan increased Ca2+ signals in dissociated DRG neurons, although it is possible other cell types contribute in vivo.

Several studies have investigated the role of TLR4 in itch. Human β-defensin 2 (hBD2) is an antimicrobial peptide highly upregulated in keratinocytes in psoriasis patients (Jansen et al. 2009). Intradermal hBD2 injection prompted itch behaviors in WT mice, but not in mice lacking Tlr4 globally or mice lacking Tlr4 selectively in myeloid cells (LysM-Cre; Tlr4fx/fx mice) Similarly, TRPV1 KO mice exhibited reduced scratching behaviors, suggesting both TLR4 and TRPV1 are important in

this process. Calcium imaging experiments revealed that hBD2 induced Ca2+ signals in keratinocytes, but not DRG sensory neurons, and this effect was TLR4-dependent. Thus, hBD2 appears to induce itch via neuro–immune interactions, accomplished via TLR4 activation in cutaneous immune cells, likely leading to induction of an unidentified pruriceptive mediator that acts, in part, via TRPV1+ in nociceptors (Feng et al. 2017). Liu et al. (2016) found that TLR4 does not contribute to acute itch behaviors induced by intradermal administration of pruritogens, but TLR4 is critical in the pathogenesis of dry skin-induced chronic itch (Tong Liu et al. 2016). Interestingly, this was attributed to TLR4 signaling in astrocytes, which contribute to central sensitization in chronic pain and chronic itch conditions (Ji et al. 2019; Liu et al. 2016).

8.9 Conclusion

Over the course of the past 40 years, our knowledge of TLRs has significantly expanded. Ample evidence now exists which demonstrates that canonical TLR signaling in immune cells is a critical regulator of pain and itch, accomplished by TLR-mediated induction of IFMs which themselves alter the excitability of peripheral nociceptors. In addition, TLR signaling in peripheral sensory neurons, including nociceptors (TLR3, TLR4, TLR7) and mechanoreceptors (TLR5), can directly modulate neuronal excitability through a unique, non-canonical, and transcription-independent mechanism involving coupling with ion channels. The plethora of research focused on how TLR signaling controls sensory neuron activity has led to a deeper understanding of the intricate coupling between sensory neurons and immune cells and the inextricable link between pain and inflammation. While less extensive research exists regarding the role of TLRs in itch, there has nevertheless been significant findings to further understand how TLRs affect acute and chronic itch conditions. Understanding how exogenous PAMPs or endogenous DAMPs signal via TLRs via canonical and non-canonical mechanisms has also altered our conceptual understanding of the role of peripheral sensory neurons as detectors of danger signals, expanding the repertoire beyond just the detection of physical and thermal stimuli. This has opened the door to an emerging, but at present undeveloped field of research focused on interactions between neurons and microorganisms. Thus, from the discovery of Toll in drosophila to recent studies investigating the role of TLRs beyond immunity and in nociception, there have been important advancements made toward a deeper understanding of TLRs in neuro–immune interactions.

References

Ahn H, Kim JM, Lee K, Kim H, Jeong D. Extracellular acidosis accelerates bone resorption by enhancing osteoclast survival, adhesion, and migration. Biochem Biophys Res Commun. (1090–2104 (Electronic)). 2012;418:144–8.

Akira S, Hoshino K. Myeloid differentiation factor 88—dependent and—independent pathways in toll-like receptor signaling. J Infect Dis. 2003;187(Supplement_2):S356–63.

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-κB by toll-like receptor 3. Nature. 2001;413(6857):732–8.

Anderson KV, Bokla L, Nüsslein-Volhard C. Establishment of dorsal-ventral polarity in the drosophila embryo: the induction of polarity by the toll gene product. Cell. 1985a;42(3):791–8.

Anderson KV, Jürgens G, Nüsslein-Volhard C. Establishment of dorsal-ventral polarity in the drosophila embryo: genetic studies on the role of the toll gene product. Cell. 1985b;42(3):779–89.

Aravalli RN, Peterson PK, Lokensgard JR. Toll-like receptors in defense and damage of the central nervous system. J Neuroimmune Pharmacol. 2007;2(4):297–312.

Augustin R, Schröder K, Murillo Rincón AP, Fraune S, Anton-Erxleben F, Herbst E-M, Wittlieb J, Schwentner M, Grötzinger J, Wassenaar TM, et al. A secreted antibacterial neuropeptide shapes the microbiome of hydra. Nat Commun. 2017;8(1):698.

Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. Annu Rev Immunol. 1994;12(1):141–79.

Barajon I, Serrao G, Arnaboldi F, Opizzi E, Ripamonti G, Balsari A, Rumio C. Toll-like receptors 3, 4, and 7 are expressed in the enteric nervous system and dorsal root ganglia. J Histochem Cytochem. 2009;57(11):1013–23.

Barragán-Iglesias P, Franco-Enzástiga Ú, Jeevakumar V, Shiers S, Wangzhou A, Granados-Soto V, Campbell ZT, Dussor G, Price TJ. Type I interferons act directly on nociceptors to produce pain sensitization: implications for viral infection-induced pain. J Neurosci. 2020;40(18):3517–32.

Bautista DM, Jordt S-E, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell. 2006;124(6):1269–82.

Belvin MP, Anderson KV. A conserved signaling pathway: the drosophila toll-dorsal pathway. Annu Rev Cell Dev Biol. 1996;12(1):393–416.

Bettoni I, Comelli F, Rossini C, Granucci F, Giagnoni G, Peri F, Costa B. 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;56(12):1312–9.

Beutler B, Rietschel ET. Innate immune sensing and its roots: the story of endotoxin. Nat Rev Immunol. 2003;3(2):169–76.

Biragyn A, Ruffini PA, Leifer CA, Klyushnenkova E, Shakhov A, Chertov O, Shirakawa AK, Farber JM, Segal DM, Oppenheim JJ, et al. Toll-like receptor 4-dependent activation of dendritic cells by β-defensin 2. Science. 2002;298(5595):1025.

Botos I, Segal DM, Davies DR. The structural biology of toll-like receptors. Structure. 2011;19(4):447–59.

Bruno K, Woller SA, Miller YI, Yaksh TL, Wallace M, Beaton G, Chakravarthy K. Targeting toll-like receptor-4 (TLR4)-an emerging therapeutic target for persistent pain states. Pain. 2018;159(10):1908–15.

Bsibsi M, Ravid R, Gveric D, van Noort JM. Broad expression of toll-like receptors in the human central nervous system. J Neuropathol Exp Neurol. 2002;61(11):1013–21.

Burnet FM. A modification of Jerne's theory of antibody production using the concept of clonal selection. CA Cancer J Clin. 1976;26(2):119–21.

Campbell JN, Raja SN, Meyer RA, Mackinnon SE. Myelinated afferents signal the hyperalgesia associated with nerve injury. Pain. 1988;32(1):89–94.

Cao X, Aballay A. Neural inhibition of dopaminergic signaling enhances immunity in a cell-non-autonomous manner. Curr Biol. 2016;26(17):2329–34.

Carpentier PA, Begolka WS, Olson JK, Elhofy A, Karpus WJ, Miller SD. Differential activation of astrocytes by innate and adaptive immune stimuli. Glia. 2005;49(3):360–74.
Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science. 2000;288(5464):306.
Chang C, Liu H-K, Yeh C-B, Yang M-L, Liao W-C, Liu C-H, Tseng T-J. Cross-talk of toll-like receptor 5 and mu-opioid receptor attenuates chronic constriction injury-induced mechanical hyperalgesia through a protein kinase C alpha-dependent signaling. Int J Mol Sci. 2021;22(4):1891.
Chen W, Lu Z. Upregulated TLR3 promotes neuropathic pain by regulating autophagy in rat with l5 spinal nerve ligation model. Neurochem Res. 2017;42(2):634–43.
Chen G, Zhang Y-Q, Qadri YJ, Serhan CN, Ji R-R. Microglia in pain: detrimental and protective roles in pathogenesis and resolution of pain. Neuron. 2018;100(6):1292–311.
Chen O, Donnelly CR, Ji R-R. Regulation of pain by neuro-immune interactions between macrophages and nociceptor sensory neurons. Curr Opin Neurobiol. 2020;62:17–25.
Chen Y, Wang Z-L, Yeo M, Zhang Q-J, López-Romero AE, Ding H-P, Zhang X, Zeng Q, Morales-Lázaro SL, Moore C, et al. Epithelia-sensory neuron cross talk underlies cholestatic itch induced by lysophosphatidylcholine. Gastroenterology. 2021;161(1):301–17.e316.
Chiu IM. Infection, pain, and itch. Neurosci Bull. 2018;34(1):109–19.
Clark AK, Staniland AA, Marchand F, Kaan TKY, McMahon SB, Malcangio M. P2X7-dependent release of interleukin-1beta and nociception in the spinal cord following lipopolysaccharide. J Neurosci. 2010;30(2):573–82.
Cristina Lebre M, Antons JC, Kalinski P, Schuitemaker JHN, van Capel TMM, Kapsenberg ML, de Jong EC. Double-stranded RNA-exposed human keratinocytes promote Th1 responses by inducing a type-1 polarized phenotype in dendritic cells: Role of keratinocyte-derived tumor necrosis factor α, type I interferons, and interleukin-18. J Investig Dermatol. 2003;120(6):990–7.
Diebold Sandra S, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 2004;303(5663):1529–31.
Diogenes A, Ferraz CC, Akopian AN, Henry MA, Hargreaves KM. LPS sensitizes TRPV1 via activation of TLR4 in trigeminal sensory neurons. J Dent Res. 2011;60:759–64. (1544-0591 (Electronic))
Dong X, Dong X. Peripheral and central mechanisms of itch. Neuron. 2018;98(3):482–94.
Donnelly CR, Chen O, Ji RR. How do sensory neurons sense danger signals? Trends in Neuroscience. 2020;43(10):822–38. (1878-108X (Electronic))
Emmerich CH, Ordureau A, Strickson S, Arthur JSC, Pedrioli PGA, Komander D, Cohen P. Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. Proc Natl Acad Sci U S A. 2013;110(38):15247–52.
Everts B, Amiel E, Huang SC-C, Smith AM, Chang C-H, Lam WY, Redmann V, Freitas TC, Blagih J, van der Windt GJW, et al. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. Nat Immunol. 2014;15(4):323–32.
Ewald SE, Engel A, Lee J, Wang M, Bogyo M, Barton GM. Nucleic acid recognition by toll-like receptors is coupled to stepwise processing by cathepsins and asparagine endopeptidase. J Exp Med. 2011;208(4):643–51.
Feng J, Luo J, Mack MR, Yang P, Zhang F, Wang G, Gong X, Cai T, Mei Z, Kim BS, et al. The antimicrobial peptide human beta-defensin 2 promotes itch through toll-like receptor 4 signaling in mice. J Allergy Clin Immunol. 2017;140(3):885–8.e886.
Fitzgerald KA, Kagan JC. Toll-like receptors and the control of immunity. Cell. 2020;180(6):1044–66.
Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT, Coyle AJ, Liao S-M, Maniatis T. IKKε and TBK1 are essential components of the IRF3 signaling pathway. Nat Immunol. 2003a;4(5):491–6.
Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E, Monks B, Pitha PM, Golenbock DT. LPS-TLR4 signaling to IRF-3/7 and NF-κB involves the toll adapters TRAM and TRIF. J Exp Med. 2003b;198(7):1043–55.

Fraune S, Bosch TCG. Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. Proc Natl Acad Sci. 2007;104(32):13146.

Fukui R, Yamamoto C, Matsumoto F, Onji M, Shibata T, Murakami Y, Kanno A, Hayashi T, Tanimura N, Yoshida N, et al. Cleavage of toll-like receptor 9 ectodomain is required for in vivo responses to single strand DNA. Front Immunol. 2018;9:1491.

Gao W, Xiong Y, Li Q, Yang H. Inhibition of toll-like receptor signaling as a promising therapy for inflammatory diseases: a journey from molecular to nano therapeutics. Front Physiol. 2017;8:508.

Gay NJ, Keith FJ. Drosophila toll and IL-1 receptor. Nature. 1991;351(6325):355–6.

Gay NJ, Symmons MF, Gangloff M, Bryant CE. Assembly and localization of toll-like receptor signalling complexes. Nat Rev Immunol. 2014;14(8):546–58.

Gewirtz AT, Navas TA, Lyons S, Godowski PJ, Madara JL. Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. J Immunol. 2001;167(4):1882.

Goethals S, Ydens E, Timmerman V, Janssens S. Toll-like receptor expression in the peripheral nerve. Glia. 2010;58(14):1701–9. (1098-1136 (Electronic))

Gorina R, Font-Nieves M, Márquez-Kisinousky L, Santalucia T, Planas AM. Astrocyte TLR4 activation induces a proinflammatory environment through the interplay between MyD88-dependent NFκB signaling, MAPK, and Jak1/Stat1 pathways. Glia. 2011;59(2):242–55.

Harvey Lodish AB, Lawrence Zipursky S, Matsudaira P, Baltimore D, Darnell J. Molecular cell biology. 4th ed. New York: W.H. Freeman; 2000.

Hasel P, Rose IVL, Sadick JS, Kim RD, Liddelow SA. Neuroinflammatory astrocyte subtypes in the mouse brain. Nat Neurosci. 2021;24:1475–87.

Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A. The innate immune response to bacterial flagellin is mediated by toll-like receptor 5. Nature. 2001;410:1099–103. (0028-0836 (Print))

Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, et al. A toll-like receptor recognizes bacterial DNA. Nature. 2000;408(6813):740–5.

Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, Horiuchi T, Tomizawa H, Takeda K, Akira S. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol. 2002;3(2):196–200.

Hodgkin PD, Heath WR, Baxter AG. The clonal selection theory: 50 years since the revolution. Nat Immunol. 2007;8(10):1019–26.

Huang J, Gandini MA, Chen L, M'Dahoma S, Stemkowski PL, Chung H, Muruve DA, Zamponi GW. Hyperactivity of innate immunity triggers pain via TLR2-IL-33-mediated neuroimmune crosstalk. Cell Rep. 2020;33(1):108233.

Ifuku M, Hinkelmann L, Kuhrt LD, Efe IE, Kumbol V, Buonfiglioli A, Krüger C, Jordan P, Fulde M, Noda M, et al. Activation of toll-like receptor 5 in microglia modulates their function and triggers neuronal injury. Acta Neuropathol Commun. 2020;8(1):159.

Ip YT, Reach M, Engstrom Y, Kadalayil L, Cai H, González-Crespo S, Tatei K, Levine M. Dif, a dorsal-related gene that mediates an immune response in drosophila. Cell. 1993;75(4):753–63.

Irazoqui JE, Troemel ER, Feinbaum RL, Luhachack LG, Cezairliyan BO, Ausubel FM. Distinct pathogenesis and host responses during infection of C. elegans by p. aeruginosa and s. aureus. PLOS Pathog. 2010a;6(7):e1000982.

Irazoqui JE, Urbach JM, Ausubel FM. Evolution of host innate defence: insights from caenorhabditis elegans and primitive invertebrates. Nat Rev Immunol. 2010b;10(1):47–58.

Janeway CA. Pillars article: approaching the asymptote? Evolution and revolution in immunology. Cold spring harb symp quant biol. J Immunol. 2013;191(9):4475.

Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol. 1989;54(Pt 1):1–13. (0091-7451 (Print))

Jansen PAM, Rodijk-Olthuis D, Hollox EJ, Kamsteeg M, Tjabringa GS, de Jongh GJ, van Vlijmen-Willems IMJJ, Bergboer JGM, van Rossum MM, de Jong EMGJ, et al. Beta-defensin-2 protein is a serum biomarker for disease activity in psoriasis and reaches biologically relevant concentrations in lesional skin. PLoS One. 2009;4(3):e4725.

Ji R-R. Neuroimmune interactions in itch: do chronic itch, chronic pain, and chronic cough share similar mechanisms? Pulm Pharmacol Ther. 2015;35:81–6.
Ji R-R, Donnelly CR, Nedergaard M. Astrocytes in chronic pain and itch. Nat Rev Neurosci. 2019;20(11):667–85.
Jin MS, Lee J-O. Structures of the toll-like receptor family and its ligand complexes. Immunity. 2008;29(2):182–91.
Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik S-G, Lee H, Lee J-O. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell. 2007;130(6):1071–82.
Kanno A, Tanimura N, Ishizaki M, Ohko K, Motoi Y, Onji M, Fukui R, Shimozato T, Yamamoto K, Shibata T, et al. Targeting cell surface TLR7 for therapeutic intervention in autoimmune diseases. Nat Commun. 2015;6(1):6119.
Karikó K, Ni H, Capodici J, Lamphier M, Weissman D. mRNA is an endogenous ligand for toll-like receptor 3. J Biol Chem. 2004;279(13):12542–50.
Kawai T, Takeuchi O, Fujita T, Inoue J-i, Mühlradt PF, Sato S, Hoshino K, Akira S. Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. J Immunol. 2001;167(10):5887.
Kelly MG, Alvero AB, Chen R, Silasi D-A, Abrahams VM, Chan S, Visintin I, Rutherford T, Mor G. Tlr-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. Cancer Res. 2006;66(7):3859.
Kido-Nakahara M, Buddenkotte J, Kempkes C, Ikoma A, Cevikbas F, Akiyama T, Nunes F, Seeliger S, Hasdemir B, Mess C, et al. Neural peptidase endothelin-converting enzyme 1 regulates endothelin 1-induced pruritus. J Clin Invest. 2014;124(6):2683–95.
Kieser KJ, Kagan JC. Multi-receptor detection of individual bacterial products by the innate immune system. Nat Rev Immunol. 2017;17(6):376–90.
Kim D, Kim MA, Cho I-H, Kim MS, Lee S, Jo E-K, Choi S-Y, Park K, Kim JS, Akira S, et al. A critical role of toll-like receptor 2 in nerve injury-induced spinal cord glial cell activation and pain hypersensitivity. J Biol Chem. 2007;282(20):14975–83.
Kim D, You B, Lim H, Lee SJ. Toll-like receptor 2 contributes to chemokine gene expression and macrophage infiltration in the dorsal root ganglia after peripheral nerve injury. Mol Pain. 2011;7:74.
Klimovich A, Giacomello S, Björklund Å, Faure L, Kaucka M, Giez C, Murillo-Rincon AP, Matt A-S, Willoweit-Ohl D, Crupi G, et al. Prototypical pacemaker neurons interact with the resident microbiota. Proc Natl Acad Sci. 2020;117(30):17854.
Kremer AE, Mettang T, Weisshaar E. Non-dermatological challenges of chronic itch. Acta Derm Venereol. 2020;100:adv00025. (1651-2057 (Electronic))
Kupari J, Usoskin D, Parisien M, Lou D, Hu Y, Fatt M, Lönnerberg P, Spångberg M, Eriksson B, Barkas N, et al. Single cell transcriptomics of primate sensory neurons identifies cell types associated with chronic pain. Nat Commun. 2021;12(1):1510.
Kurban MS, Boueiz A, Kibbi A-G. Cutaneous manifestations of chronic kidney disease. Clin Dermatol. 2008;26(3):255–64.
Lacagnina MJ, Watkins LR, Grace PM. Toll-like receptors and their role in persistent pain. Pharmacol Ther. 2018;184:145–58.
Lafon M, Megret F, Lafage M, Prehaud C. The innate immune facet of brain. J Mol Neurosci. 2006;29(3):185–94.
Latz E, Verma A, Visintin A, Gong M, Sirois CM, Klein DCG, Monks BG, McKnight CJ, Lamphier MS, Duprex WP, et al. Ligand-induced conformational changes allosterically activate toll-like receptor 9. Nat Immunol. 2007;8(7):772–9.
Lebre MC, van der Aar AMG, van Baarsen L, van Capel TMM, Schuitemaker JHN, Kapsenberg ML, de Jong EC. Human keratinocytes express functional toll-like receptor 3, 4, 5, and 9. J Investig Dermatol. 2007;127(2):331–41.
Li Y, Zhang H, Zhang H, Kosturakis AK, Jawad AB, Dougherty PM. Toll-like receptor 4 signaling contributes to paclitaxel-induced peripheral neuropathy. J Pain. 2014;15(7):712–25.

Liu Q, Ding JL. The molecular mechanisms of TLR-signaling cooperation in cytokine regulation. Immunol Cell Biol. 2016;94(6):538–42.
Liu B, Yang Y, Qiu Z, Staron M, Hong F, Li Y, Wu S, Li Y, Hao B, Bona R, et al. Folding of toll-like receptors by the HSP90 paralogue gp96 requires a substrate-specific cochaperone. Nat Commun. 2010a;1(1):79.
Liu T, Xu Z-Z, Park C-K, Berta T, Ji R-R. Toll-like receptor 7 mediates pruritus. Nat Neurosci. 2010b;13(12):1460–2.
Liu T, Berta T, Xu ZZ, Park CK, Zhang L, Lü N, Liu Q, Liu Y, Gao YJ, Liu YC, et al. TLR3 deficiency impairs spinal cord synaptic transmission, central sensitization, and pruritus in mice. J Clin Investig. 2012a;122(6):2195–207.
Liu T, Gao YJ, Ji R-R. Emerging role of toll-like receptors in the control of pain and itch. Neurosci Bull. 2012b;28(2):131–44. (1995-8218 (Electronic))
Liu X-J, Zhang Y, Liu T, Xu Z-Z, Park C-K, Berta T, Jiang D, Ji R-R. Nociceptive neurons regulate innate and adaptive immunity and neuropathic pain through myd88 adapter. Cell Res. 2014;24(11):1374–7.
Liu T, Han Q, Chen G, Huang Y, Zhao L-X, Berta T, Gao Y-J, Ji R-R. Toll-like receptor 4 contributes to chronic itch, alloknesis, and spinal astrocyte activation in male mice. Pain. 2016;157(4):806–17.
Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2:17023.
Luo X, Huh Y, Bang S, He Q, Zhang L, Matsuda M, Ji R-R. Macrophage toll-like receptor 9 contributes to chemotherapy-induced neuropathic pain in male mice. J Neurosci. 2019;39(35):6848–64.
Ma Y, Haynes RL, Sidman RL, Vartanian T. TLR8: an innate immune receptor in brain, neurons and axons. Cell Cycle. 2007;6(23):2859–68.
Madan V, Lear JT, Szeimies R-M. Non-melanoma skin cancer. Lancet. 2010;375(9715):673–85.
Materazzi S, Fusi C, Benemei S, Pedretti P, Patacchini R, Nilius B, Prenen J, Creminon C, Geppetti P, Nassini R. TRPA1 and TRPV4 mediate paclitaxel-induced peripheral neuropathy in mice via a glutathione-sensitive mechanism. Pflugers Arch. 2012;463(4):561–9.
McKimmie CS, Fazakerley JK. In response to pathogens, glial cells dynamically and differentially regulate toll-like receptor gene expression. J Neuroimmunol. 2005;169(1):116–25.
McWhirter SM, Fitzgerald KA, Rosains J, Rowe DC, Golenbock DT, Maniatis T. IFN-regulatory factor 3-dependent gene expression is defective in TBK1-deficient mouse embryonic fibroblasts. Proc Natl Acad Sci. 2004;101(1):233.
Medzhitov R, Preston-Hurlburt P, Janeway CA. A human homologue of the drosophila toll protein signals activation of adaptive immunity. Nature. 1997;388(6640):394–7.
Mielcarska MB, Bossowska-Nowicka M, Toka FN. Cell surface expression of endosomal toll-like receptors-A necessity or a superfluous duplication? Front Immunol. 2021;11:620972.
Miller LS. Toll-like receptors in skin. Adv Dermatol. 2008;24:71–87.
Min H, Cho W-H, Lee H, Choi B, Kim Y-J, Lee HK, Joo Y, Jung SJ, Choi S-Y, Lee S, et al. Association of TRPV1 and TLR4 through the TIR domain potentiates TRPV1 activity by blocking activation-induced desensitization. Mol Pain. 2018;14:1744806918812636.
Modlin RL. Innate immunity: ignored for decades, but not forgotten. J Invest Dermatol. 2012;132(3 Pt 2):882–6.
Mohammad Hosseini A, Majidi J, Baradaran B, Yousefi M. Toll-like receptors in the pathogenesis of autoimmune diseases. Adv Pharm Bull. 2015;5(Suppl 1):605–14.
Moore C, Gupta R, Jordt S-E, Chen Y, Liedtke WB. Regulation of pain and itch by TRP channels. Neurosci Bull. 2018;34(1):120–42.
Navarro L, David M. P38-dependent activation of interferon regulatory factor 3 by lipopolysaccharide. J Biol Chem. 1999;274(50):35535–8.
Nicotra L, Loram LC, Watkins LR, Hutchinson MR. Toll-like receptors in chronic pain. Exp Neurol. 2012;234(2):316–29.
Ohsawa M, Miyabe Y, Katsu H, Yamamoto S, Ono H. Identification of the sensory nerve fiber responsible for lysophosphatidic acid-induced allodynia in mice. Neuroscience. 2013;247:65–74.

Ohto U, Ishida H, Shibata T, Sato R, Miyake K, Shimizu T. Toll-like receptor 9 contains two DNA binding sites that function cooperatively to promote receptor dimerization and activation. Immunity. 2018;48(4):649–58.e644.

Olson JK, Miller SD. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol. 2004;173(6):3916.

Ossipov MH, Zhang E-T, Carvajal C, Gardell L, Quirion R, Dumont Y, Lai J, Porreca F. Selective mediation of nerve injury-induced tactile hypersensitivity by neuropeptide y. J Neurosci. 2002;22(22):9858–67.

Park BS, Song DH, Kim HM, Choi B-S, Lee H, Lee J-O. The structural basis of lipopolysaccharide recognition by the TLR4–MD-2 complex. Nature. 2009;458(7242):1191–5.

Park C-K, Xu Z-Z, Berta T, Han Q, Chen G, Liu X-J, Ji R-R. Extracellular microRNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. Neuron. 2014;82(1):47–54.

Parkin J, Cohen B. An overview of the immune system. Lancet. 2001;357(9270):1777–89.

Patapoutian A, Tate S, Woolf CJ. Transient receptor potential channels: targeting pain at the source. Nat Rev Drug Discov. 2009;8(1):55–68.

Pinho-Ribeiro FA, Verri WA Jr, Chiu IM. Nociceptor sensory neuron-immune interactions in pain and inflammation. Trends Immunol. 2017;38(1):5–19.

Pujol N, Link EM, Liu LX, Kurz CL, Alloing G, Tan M-W, Ray KP, Solari R, Johnson CD, Ewbank JJ. A reverse genetic analysis of components of the toll signaling pathway in caenorhabditis elegans. Curr Biol. 2001;11(11):809–21.

Qi J, Buzas K, Fan H, Cohen JI, Wang K, Mont E, Klinman D, Oppenheim JJ, Howard OMZ. Painful pathways induced by TLR stimulation of dorsal root ganglion neurons. J Immunol. 2011;186(11):6417–26.

Randow F, Seed B. Endoplasmic reticulum chaperone gp96 is required for innate immunity but not cell viability. Nat Cell Biol. 2001;3(10):891–6.

Ren K, Dubner R. Interactions between the immune and nervous systems in pain. Nat Med. 2010;16(11):1267–76.

Renthal W, Tochitsky I, Yang L, Cheng Y-C, Li E, Kawaguchi R, Geschwind DH, Woolf CJ. Transcriptional reprogramming of distinct peripheral sensory neuron subtypes after axonal injury. Neuron. 2020;108(1):128–44.e129.

Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF. A family of human receptors structurally related to drosophila toll. Proc Natl Acad Sci U S A. 1998;95(2):588–93.

Saito O, Svensson CI, Buczynski MW, Wegner K, Hua XY, Codeluppi S, Schaloske RH, Deems RA, Dennis EA, Yaksh TL. Spinal glial TLR4-mediated nociception and production of prostaglandin E(2) and TNF. Br J Pharmacol. 2010;160(7):1754–64.

Scumpia PO, Kelly KM, Reeves WH, Stevens BR. Double-stranded RNA signals antiviral and inflammatory programs and dysfunctional glutamate transport in TLR3-expressing astrocytes. Glia. 2005;52(2):153–62.

Seong S-Y, Matzinger P. Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. Nat Rev Immunol. 2004;4(6):469–78.

Shen S, Lim G, You Z, Ding W, Huang P, Ran C, Doheny J, Caravan P, Tate S, Hu K, et al. Gut microbiota is critical for the induction of chemotherapy-induced pain. Nat Neurosci. 2017;20(9):1213–6.

Shi XQ, Zekki H, Zhang J. The role of TLR2 in nerve injury-induced neuropathic pain is essentially mediated through macrophages in peripheral inflammatory response. Glia. 2011;59(2):231–41.

Shimizu Y, Sonoda A, Nogi C, Ogushi Y, Kanda R, Yamaguchi S, Nohara N, Aoki T, Yamada K, Nakata J, et al. B-type (brain) natriuretic peptide and pruritus in hemodialysis patients. Int J Nephrol Renovasc Dis. 2014;7:329–35.

Sims JE, March CJ, Cosman D, Widmer MB, MacDonald HR, McMahan CJ, Grubin CE, Wignall JM, Jackson JL, Call SM, et al. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. Science. 1988;241(4865):585.

Steward R. Dorsal, an embryonic polarity gene in drosophila, is homologous to the vertebrate proto-oncogene, c-rel. Science. 1987;238(4827):692.
Su X-Y, Chen M, Yuan Y, Li Y, Guo S-S, Luo H-Q, Huang C, Sun W, Li Y, Zhu MX, et al. Central processing of itch in the midbrain reward center. Neuron. 2019;102(4):858–72.e855.
Szabo-Pardi TA, Barron LR, Lenert ME, Burton MD. Sensory neuron TLR4 mediates the development of nerve-injury induced mechanical hypersensitivity in female mice. Brain Behav Immun. 2021;97:42–60.
Szöllősi AG, McDonald I, Szabó IL, Meng J, van den Bogaard E, Steinhoff M. TLR3 in chronic human itch: a keratinocyte-associated mechanism of itch sensitization. J Investig Dermatol. 2019;139(11):2393–6.e2396.
Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010;140(6):805–20.
Tan Y, Kagan JC. Innate immune signaling organelles display natural and programmable signaling flexibility. Cell. 2019;177(2):384–98.e311.
Tan Y, Zanoni I, Cullen TW, Goodman AL, Kagan JC. Mechanisms of toll-like receptor 4 endocytosis reveal a common immune-evasion strategy used by pathogenic and commensal bacteria. Immunity. 2015;43(5):909–22.
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 U S A. 2005;102(16):5856–61.
Tanji H, Ohto U, Motoi Y, Shibata T, Miyake K, Shimizu T. Autoinhibition and relief mechanism by the proteolytic processing of toll-like receptor 8. Proc Natl Acad Sci. 2016;113(11):3012.
Tse KH, Chow KBS, Leung WK, Wong YH, Wise H. Primary sensory neurons regulate toll-like receptor-4-dependent activity of glial cells in dorsal root ganglia. Neuroscience. 2014;279:10–22.
Usoskin D, Furlan A, Islam S, Abdo H, Lönnerberg P, Lou D, Hjerling-Leffler J, Haeggström J, Kharchenko O, Kharchenko PV, et al. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. Nat Neurosci. 2015;18(1):145–53.
Wang C, Deng L, Hong M, Akkaraju GR, Inoue J-i, Chen ZJ. Tak1 is a ubiquitin-dependent kinase of MKK and IKK. Nature. 2001;412(6844):346–51.
Wang T-T, Xu X-Y, Lin W, Hu D-D, Shi W, Jia X, Wang H, Song N-J, Zhang Y-Q, Zhang L. Activation of different heterodimers of TLR2 distinctly mediates pain and itch. Neuroscience. 2020;429:245–55.
Wani KA, Goswamy D, Irazoqui JE. Nervous system control of intestinal host defense in C. elegans. Curr Opin Neurobiol. 2020;62:1–9.
Whitham S, McCormick S, Baker B. The N gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. Proc Natl Acad Sci U S A. 1996;93(16):8776–81.
Wu F-x, Bian J-j, Miao X-r, Huang S-d, Xu X-w, Gong D-j, Sun Y-m, Lu Z-j, Yu W-f. Intrathecal siRNA against toll-like receptor 4 reduces nociception in a rat model of neuropathic pain. Int J Med Sci. 2010;7(5):251–9.
Xu Z-Z, Kim YH, Bang S, Zhang Y, Berta T, Wang F, Oh SB, Ji R-R. Inhibition of mechanical allodynia in neuropathic pain by TLR5-mediated A-fiber blockade. Nat Med. 2015;21(11):1326–31.
Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science. 2003a;301(5633):640.
Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshino K, Kaisho T, Takeuchi O, Takeda K, Akira S. Tram is specifically involved in the toll-like receptor 4–mediated MyD88-independent signaling pathway. Nat Immunol. 2003b;4(11):1144–50.
Yang Z, Liu P, Bai L, Trimmer JS, Bean BP, Ginty DD. Deep sequencing of somatosensory neurons reveals molecular determinants of intrinsic physiological properties. Neuron. 2019;103(4):598–616.e597.
Zanoni I, Ostuni R, Marek Lorri R, Barresi S, Barbalat R, Barton Gregory M, Granucci F, Kagan Jonathan C. CD14 controls the LPS-induced endocytosis of toll-like receptor 4. Cell. 2011;147(4):868–80.

Zeisel A, Hochgerner H, Lönnerberg P, Johnsson A, Memic F, van der Zwan J, Häring M, Braun E, Borm LE, La Manno G, et al. Molecular architecture of the mouse nervous system. Cell. 2018;174(4):999–1014.e1022.

Zhang Z-J, Guo J-S, Li S-S, Wu X-B, Cao D-L, Jiang B-C, et al. TLR8 and its endogenous ligand miR-21 contribute to neuropathic pain in murine DRG. J Exp Med. 2018;215(12):3019–3037. https://doi.org/10.1084/jem.20180800. PMID 30455267.

Zhao L-X, Jiang M, Bai X-Q, Cao D-L, Wu X-B, Zhang J, et al. (2021). TLR8 in the trigeminal ganglion contributes to the maintenance of trigeminal neuropathic pain in mice. Neurosci Bull. 2021;37(4):550–562. https://doi.org/10.1007/s12264-020-00621-4. PMID 33355900.

Zuany-Amorim C, Hastewell J, Walker C. Toll-like receptors as potential therapeutic targets for multiple diseases. Nat Rev Drug Discov. 2002;1(10):797–807. https://doi.org/10.1038/nrd914. PPMID 12360257.