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Toll-like receptors and their role in persistent pain

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Abstract

One of the fundamental mechanisms whereby the innate immune system coordinates inflammatory signal transduction is through Toll-like receptors (TLRs), which function to protect and defend the host organism by initiating inflammatory signaling cascades in response to tissue damage or injury. TLRs are positioned at the neuroimmune interface, and accumulating evidence suggests that the inflammatory consequences of TLR activation on glia (including microglia and astrocytes), sensory neurons, and other cell types can influence nociceptive processing and lead to states of exaggerated and unresolved pain. In this review, we summarize our current understanding of how different TLRs and their accessory molecules can contribute to the development and maintenance of persistent pain. The challenges and opportunities of targeting TLRs for new treatment strategies against chronic pain are discussed, including the therapeutic context of TLR-mediated signaling in opioid analgesia and chemotherapy-induced pain. Considering the prevalence of persistent pain and the insufficient efficacy and safety of current treatment options, a deeper understanding of Toll-like receptors holds the promise of novel therapies for managing pathological pain.

Keywords

pain; TLR; innate immunity; inflammation; glia; nociception

1. Introduction

Preclinical studies have identified innate immune signaling as a major mechanism responsible for persistent pain. Germline-encoded receptors and effectors that recognize a limited number of invariant molecular patterns are a key feature of the innate immune

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Conflict of interest statement

MJL and PMG declare there are no conflicts of interest. LRW is a co-founder of Xalud Therapeutics and a co-chair of the Xalud Therapeutics Scientific Advisory Committee.

system, which produce immediate and stereotyped immune responses that are integral for mounting an appropriate defense against sterile and pathogenic injuries across phylogeny (Leulier & Lemaitre, 2008). Recognition of conserved ligands by the innate immune system involves several classes of receptors collectively known as pattern recognition receptors (PRRs), which discriminate molecular sequences and can be categorized by the ligands they identify, their cellular localization, and the functional outcomes they support (Janeway & Medzhitov, 2002). The family of Toll-like receptors (TLRs) were the first PRRs to be identified and have since been widely characterized for their essential contributions to innate immune signaling (Kawai & Akira, 2011).

The discovery of TLRs originated from the identification of the protein Toll, which plays an essential role in dictating dorsoventral polarity during larval embryogenesis for the fruit fly *Drosophila melanogaster* (Anderson, Bokla, & Nüsslein-Volhard, 1985; Anderson, Jürgens, & Nüsslein-Volhard, 1985). Toll was further identified as a transmembrane interleukin-1 (IL-1) receptor (IL-1R) homolog (Gay & Keith, 1991; Hashimoto, Hudson, & Anderson, 1988) that initiates immune responses in cultured *Drosophila* hemocytes (Rosetto, Engstrom, Baldari, Telford, & Hultmark, 1995). The role of Toll as a molecular substrate of host defense was confirmed following the observation of its antifungal signaling properties in adult flies (Lemaitre, Nicolas, Michaut, Reichhart, & Hoffmann, 1996). A human homolog of *Drosophila* Toll (hence the nomenclature Toll-like) was soon cloned and characterized as a transmembrane protein capable of inducing nuclear factor-κB (NF-κB)-mediated transcription of the pro-inflammatory cytokines IL-1, IL-6, and IL-8 in human monocytes (Medzhitov, Preston-Hurlburt, & Janeway, 1997). The discovery of this receptor (later renamed TLR4) provided the initial evidence for TLRs as regulators of mammalian immunity (see O'Neill, Golenbock, & Bowie, 2013, for a detailed history). A total of 13 TLRs have since been identified between humans and rodents (humans functionally express TLR1 to TLR10, while rodents express TLR1 to TLR9, and TLR11 to TLR13).

There has been extraordinary interest in elaborating the signaling principles of TLRs to uncover their functions for maintaining tissue homeostasis, and how their dysfunction leads to detrimental outcomes ranging from unresolved inflammation to diseases of autoimmunity. Beyond their role as innate immune receptors for pathogenic invaders, there is considerable evidence that they are critical sensors of sterile, cellular injury, and coordinate nociceptive processing via inflammatory signaling. Here, we summarize the preclinical evidence and propose a framework for the contribution of TLR signaling and neuroimmune crosstalk in mediating persistent pain. Understanding the unique and shared contributions of TLRs in the development and maintenance of persistent pain may reveal promising opportunities for improving pain management.

2. Overview of pain neurobiology

Acute pain is protective and adaptive, warning the organism to escape danger, and to protect the site of tissue injury during healing. Such painful stimuli (e.g. mechanical, thermal and chemical) are initially transduced by a range of ion channels and G-protein coupled receptors that are expressed at peripheral nociceptor terminals (Basbaum, Bautista, Scherrer, & Julius, 2009; Peirs & Seal, 2016). The action potential is conducted along these first order

nociceptive neurons, and transmitted at central synapses via neurotransmitters and neuropeptides that have the potential to excite a complex network of second-order nociceptive projection neurons in the spinal and medullary dorsal horns (Peirs & Seal, 2016). Second-order nociceptive projection neurons project to supra-spinal sites, such as thalamic nuclei, which further project to cortical and subcortical regions via third-order neurons, enabling the encoding and perception of the multidimensional pain experience (Wiech, 2016). These nociceptive signals may be suppressed in the spinal cord through activation of local inhibitory interneurons that produce γ-aminobutyric acid (GABA) or glycine, and via descending serotonergic and noradrenergic projections from brainstem sites to the spinal cord. Such modulatory pathways serve to influence the response to and perception of pain (Ossipov, Dussor, & Porreca, 2010; Peirs & Seal, 2016).

However, pain can also persist well beyond the resolution of the initial injury, as a consequence of ongoing inflammation (e.g. rheumatoid arthritis), or after damage to the nervous system (neuropathic pain). Persistent pain can also develop where a precipitating noxious stimulus is not well characterized – such as fibromyalgia or irritable bowel syndrome – (dysfunctional pain) and is poorly understood. Neuropathic and dysfunctional pain are believed to be the consequence of amplified sensory signals in the peripheral and central nervous systems. The underlying mechanisms of such sensitization have been extensively studied (Gold & Gebhart, 2010; Kuner, 2010; Latremoliere & Woolf, 2009), facilitated by a vast array of rodent pain models (see Mogil, 2009). These investigations have revealed that sensitization is not solely driven by direct neuronal communication, but rather by crosstalk between neurons and non-neuronal cells, such as glia, leukocytes and keratinocytes.

3. Neuroimmune contribution to sensitization underlying persistent pain

In response to high-threshold activity or damage of first-order neurons, infiltrating immune cells and resident non-neuronal cells are activated at peripheral nociceptor terminals, at the dorsal root ganglia (DRG) containing sensory neuron cell bodies, and at the spinal cord dorsal horn (Grace, Hutchinson, Maier, & Watkins, 2014; Ji, Chamessian, & Zhang, 2016; Old, Clark, & Malcangio, 2015). A consequence of non-neuronal cell activation is release of immune mediators that promote peripheral and central sensitization through neuromodulation and dysfunctional synaptic plasticity. For example, pro-inflammatory cytokines like tumor necrosis factor (TNF) and IL-1β, as well as nitroxidative species enhance excitatory neurotransmission by mechanisms including neurotransmitter exocytosis, and increased synaptic strength (Gwak, Hassler, & Hulsebosch, 2013; Kawasaki, Zhang, Cheng, & Ji, 2008; Kronschläger, et al., 2016; Reeve, Patel, Fox, Walker, & Urban, 2000; Yan & Weng, 2013). Glutamate homeostasis is also impaired by these mediators, which induce downregulation and post-translational modifications of glutamate transporters and glutamine synthetase (Chen, et al., 2010; Yan, Yadav, Gao, & Weng, 2014). These immune-derived molecules, together with growth factors such as brain-derived neurotrophic factor (BDNF), further promote neuroexcitability in pain pathways by disinhibiting GABAergic and glycinergic control (Coull, et al., 2005; Kawasaki, et al., 2008; Yowtak, et al., 2011; Zhang, Nei, & Dougherty, 2010). In addition to secreted factors, non-neuronal cells in the spinal cord, in particular microglia, can sculpt synaptic elements through phagocytosis of

neuronal structures, which can further disrupt the balance of excitatory and inhibitory neurotransmission encoding nociception through excessive pruning of GABAergic terminals (Batti, et al., 2016).

Noxious insults induce release of a host of neuronally-derived mediators – like adenosine triphosphate (ATP), chemokine (C-C motif) ligand 2 (CCL2), CCL21, neuregulin-1, and colony stimulating factor 1 (CSF-1) – which can activate non-neuronal cells, such as glia, via their cognate receptors (Abbadie, et al., 2003; Biber, et al., 2011; Calvo, et al., 2010; Guan, et al., 2016; Tsuda, et al., 2003). Toll-like receptor signaling has also emerged as a major pathway that mediates the activation of both neuronal and non-neuronal cells after injury and infection/inflammation. Here, we discuss the receptor subtypes responsible, their endogenous ligands that are released after injury, and the cell signaling pathways responsible for the production of soluble factors that mediate persistent pain.

4. Toll-like receptors

Pattern recognition after tissue injury

Beyond pathogen recognition, TLRs also function to recognize molecular patterns of ligands that are associated with cellular stress, tissue damage, or cell death. These noxious endogenous ligands are known as damage-associated molecular patterns (DAMPs; also known as alarmins) and they encompass a wide array of structurally diverse endogenous compounds. Many of the DAMPs identified as putative TLR ligands are molecules typically sequestered within intracellular compartments, but become liberated into the extracellular milieu during stress or tissue injury, including heat shock proteins (HSPs), high-mobility group box protein 1 (HMGB1), S100 proteins, nucleic acids, and histone proteins, among many others (Piccinini & Midwood, 2010; Trotta, Porro, Calvello, & Panaro, 2014). In addition, breakdown of the extracellular matrix caused by the injury or from injury-related protease secretion produces several DAMPs that are recognized by TLRs, including hyaluronic acid, fibrinogen, tenascin-C, and biglycan (Didangelos, et al., 2016; Piccinini & Midwood, 2010). Furthermore, oxidation of lipids from cellular membranes can also function as DAMP signaling molecules (Miller, et al., 2003). Evidence continues to accumulate that DAMP-mediated activation of TLRs are involved in initiating the sterile inflammatory response to tissue injury, which can occur in the absence of pathogen infiltration (Chen & Nuñez, 2010; Matzinger, 1994). Understanding TLR recognition of DAMPs is particularly relevant for many hypotheses regarding neuroimmune mechanisms of nociception, where some mechanical, chemical, or immunological insult that results in tissue injury almost always predates the onset of persistent pain.

Structure homology and general overview on localization and cell signaling

TLRs are type I transmembrane receptors with an N-terminal ectodomain containing a leucine-rich repeat motif, which folds into a solenoid structure to accommodate ligand recognition and accessory molecule interactions (Bell, et al., 2005; Choe, Kelker, & Wilson, 2005). Truncation of this ectodomain results in constitutive signaling, suggesting the solenoid structure is under baseline autoinhibition that is relieved by ligand interactions (Panter & Jerala, 2011). The transmembrane element extending from the ectodomain leads

to a C-terminal cytosolic Toll/interleukin-1 receptor (TIR) domain, which initiates downstream signaling processes through interactions with different adaptor proteins (Botos, Segal, & Davies, 2011). Beyond these shared structural elements, TLR signaling complexes can begin to be differentiated by their subcellular localization, which can further dictate the types of molecular patterns encountered; one group (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10) are expressed primarily on the cell surface, while the second group (TLR3, TLR7, TLR8, and TLR9, TLR11, TLR12, and TLR13) are normally expressed in intracellular compartments such as endosomes, endoplasmic reticulum, or lysosomes. Under homeostatic conditions, the cell-surface TLRs are monomeric but rapidly form homodimers or heterodimers when exposed to their cognate ligands; several of the intracellular TLRs (e.g. TLR7–TLR9), in contrast, are synthesized as receptor dimers, where ligand binding produces conformational changes at the receptor interface to bring the intracellular TIR domains into functional proximity (Gay, Symmons, Gangloff, & Bryant, 2014; Tanji, Ohto, Shibata, Miyake, & Shimizu, 2013). Differences in receptor dimerization and recruitment of unique adaptor proteins allow for further distinction between TLRs in tuning downstream signaling outcomes (Lee, Avalos, & Ploegh, 2012; O'Neill & Bowie, 2007).

TLRs are expressed by a wide variety of cells in rodents and humans (see Kato, Agalave, & Svensson, 2016); within the tissues of the nervous system, TLR expression has been identified on microglia, astrocytes, oligodendrocytes, Schwann cells, satellite glial cells, fibroblasts, endothelial cells, macrophages, and sensory neurons (Bsibsi, Ravid, Gveric, & van Noort, 2002; Goethals, Ydens, Timmerman, & Janssens, 2010; Jack, et al., 2005; Kim, You, Lim, & Lee, 2011; Laflamme & Rivest, 2001; Nagyőszi, et al., 2010; Xu, et al., 2015; Yao, et al., 2015). It is important to note that the regulation of TLR expression is highly dynamic and can differ under basal vs. inflammatory or activated conditions. Some of the experiments determining cellular expression are based on *ex vivo* preparations that can differ dramatically from physiological conditions, which could alter TLR expression or induce its transcription in cell types not expressing detectable levels at baseline. Nevertheless, the available *in vitro* and *in vivo* evidence demonstrate extensive TLR expression across many cell types. Despite their widespread expression, divergent outcomes can be elicited from TLR activation depending on its cellular localization. For instance, TLR2 expressed on bone marrow-derived inflammatory monocytes ($Ly6C^{hi}Ly6G^{-}$ cells) can produce type I interferons (IFNs) in response to vaccinia virus, while this response is absent in TLR2-expressing plasmacytoid dendritic cells (Barbalat, Lau, Locksley, & Barton, 2009). While the nature of these divergent outcomes is not entirely clear, it demonstrates that the complexity of TLR-mediated responses may involve regulatory mechanisms that differ between cells of unique ontogeny. In this capacity, cell type-specific expression of TLRs and their arrangement in distinct sub-cellular domains is likely a further determinant for generating selectivity of innate immune responses (Kawai & Akira, 2010).

While each TLR can recognize a range of molecular patterns from exogenous and endogenous ligands, there is nevertheless specificity in terms of which ligands a given TLR can recognize, which permits the generation of appropriate immune response to the corresponding pathogen or DAMP (Akira & Takeda, 2004). However, in general, ligand recognition by TLRs results in receptor dimerization, recruitment of adaptor proteins, and the induction of immune mediators (including pro-inflammatory cytokines and chemokines)

through downstream transcriptional regulation of target genes (for example, NF-κB or mitogen-activated kinases (MAPK)), to be elaborated upon in subsequent sections. The functional outcomes of PRR activation includes opsonization of pathogens, cytoskeletal reorganization leading to phagocytosis, secretion of immune mediators (e.g. pro-inflammatory cytokines), and activation of the adaptive immune system, leading to general tissue inflammation and subsequent tissue repair (Medzhitov & Janeway, 2000).

TLR-mediated neuroinflammatory signaling has been implicated in a variety of central nervous system (CNS) pathologies, including ischemic stroke, traumatic brain injury, multiple sclerosis, Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease (Buchanan, Hutchinson, Watkins, & Yin, 2010; Hanamsagar, Hanke, & Kielian, 2012; Xiang, Chao, & Feng, 2015), in addition to some neuropsychiatric diseases ranging from depression (García Bueno, Caso, Madrigal, & Leza, 2016) to drug addiction (Lacagnina, Rivera, & Bilbo, 2017). Adding to this panoply of conditions, there is also substantial evidence for the involvement of TLRs in the chronic CNS dysfunction of pathological pain. In the following sections, we will cover individual TLRs and highlight evidence associating their function with the development and maintenance of pain, and how disruption of their signaling may offer therapeutic potential for ameliorating persistent pain.

5. TLR4

TLR4 signaling

Toll-like receptor 4 (TLR4) is the most extensively characterized of the TLRs based on its fundamental role in bacterial sensing and orchestration of the resulting inflammatory response. The canonical ligand for TLR4 is lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria with a lipid moiety – known as lipid A (Strain, Fesik, & Armitage, 1983) – that produces an incredibly robust inflammatory immune response that can lead to sepsis if the immune response is not properly regulated (Roger, et al., 2009). The *in vitro* and *in vivo* immune response to LPS is dependent upon TLR4, as either spontaneous mutations in the *Tlr4* gene (i.e. the dominate negative missense mutation in C3H/HeJ mice and homozygous null mutation in C57BL/10ScCr mice) or genetic knockout of *Tlr4* (*Tlr4*$^{-/-}$ mice) renders mice nonresponsive to LPS (Hoshino, et al., 1999; Poltorak, et al., 1998; Qureshi, et al., 1999). Recognition of LPS by TLR4 is multifaceted, requiring the coordination of multiple accessory proteins and a co-receptor. Circulating LPS-binding protein (LBP) brings LPS to the cell-surface receptor cluster of differentiation 14 (CD14), a chaperone protein that delivers LPS to the TLR4 complex (Schumann, et al., 1990; Wright, Ramos, Tobias, Ulevitch, & Mathison, 1990) and dictates TLR4 receptor endocytosis (Zanoni, et al., 2011). Recognition of LPS requires the co-receptor myeloid differentiation factor 2 (MD-2), which provides a hydrophobic core that can bind the lipid A hexa-acylated chain from LPS (Shimazu, et al., 1999). MD-2 occupies a space within the solenoid structure of TLR4 and stabilizes the active LPS-bound complex, resulting in dimerization of TLR4 monomers and bringing the cytosolic TIR domains into proximity (Park, et al., 2009). Subsequent conformational changes allow for the recruitment of adaptor proteins for the initiation of intracellular signaling cascades, primarily MyD88 and TRIF. MyD88 (myeloid differentiation primary response gene 88) is utilized by TLR4 and all other

TLRs (with the exception of TLR3); its recruitment can lead to activation of NF-κB, MAPKs, activator protein-1 (AP-1), and IFN regulatory factor 5 (IRF5), culminating in the transcription of cytokines, chemokines, and other immune mediators (Arthur & Ley, 2013; Medzhitov, et al., 1998; Takaoka, et al., 2005). These include the production of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF) and inducible nitric oxide synthase (iNOS), but also anti-inflammatory IL-1R antagonist and IL-10. An alternative pathway involves recruitment of TRIF (TIR-domain-containing adaptor protein inducing interferon-β), resulting in transcription factor IRF3-dependent regulation of type I IFNs (Yamamoto, et al., 2003), the expression of which has been associated with antiviral immunity and inhibition of nociceptive transmission (Liu, Gao, et al., 2016). However, like MyD88, TRIF can result in nuclear translocation of NF-κB and cytokine transcription. Among the TLRs, TLR4 is unique in its capacity to signal through both MyD88-dependent and TRIF-dependent pathways. The mechanisms dictating how these pathways are selectively recruited is a matter of considerable interest. Although it is generally assumed that alternative TLR4 ligands engage similar signaling principles to those revealed by LPS, differential chemical or structural interactions with the TLR4/MD-2 complex may bias the inflammatory signaling.

Within the tissues of the nervous system involved in nociception, mRNA and/or protein for TLR4 is widely expressed on microglia (Zhang, et al., 2013) but has also been identified on astrocytes (Liu, Han, et al., 2016) and primary sensory neurons, in particular the capsaicin-sensitive nociceptive neurons of the trigeminal ganglia (Wadachi & Hargreaves, 2006) and the DRG that co-express transient receptor potential vanilloid 1 (TRPV1) and ATP-gated ionotropic purinergic receptor P2X3 (Helley, Abate, Jackson, Bennett, & Thompson, 2015). LPS can sensitize the firing rate of TRPV1-expressing sensory neurons to subsequent stimulation, thereby directly influencing neuronal hypersensitivity through TLR4-mediated mechanisms (Diogenes, Ferraz, Akopian, Henry, & Hargreaves, 2011; Li, et al., 2015). For immunocompetent non-neuronal cells such as microglia and astrocytes, there is agreement that the neuroimmune consequences of TLR4 activation (such as secretion of TNF or IL-1β) can indirectly influence neuronal function in the context of nociceptive signaling (Ji, et al., 2016). Additionally, while the immune mediators released from TLR4-expressing cells following receptor activation can aid in host defense, many are neurotoxic and must be quickly inactivated upon pathogen/damage clearance to prevent additional neuronal injury. Indeed, direct agonism of TLR4 can be potently neurotoxic. Treatment of cultured spinal cord cells from mouse or chicken with high-dose LPS leads to neuronal death and axonal loss, an effect that is mirrored when dorsal root ganglion cells are co-cultured with mixed glial cells (Lehnardt, et al., 2003). Removing microglia from cultures or deriving cells from the *Tlr4*-mutant C3H/HeJ mouse strain prevented the neurotoxic effects of *in vitro* LPS (Lehnardt, et al., 2003). Using improved methods to resolve the cell type-specific contribution, it is the activation and proliferation of microglia in the spinal cord (rather than infiltrating monocytes) that are largely associated with the emergence of pain hypersensitivity following peripheral nerve injury (Gu, et al., 2016). These data position glia as essential regulators of TLR4-mediated toxicity and more generally as participants in the inflammatory events that are associated with pathological pain (Milligan & Watkins, 2009).

TLR4 and persistent pain

There is accumulating evidence for TLR4 as a critical receptor facilitating inflammatory responses that are associated with the pathology of persistent pain (Buchanan, et al., 2010; DeLeo, Tanga, & Tawfik, 2004; Guo & Schluesener, 2007; Heiman, Pallottie, Heary, & Elkabes, 2014; Nicotra, Loram, Watkins, & Hutchinson, 2012). Early work in preclinical models first identified alterations in *Tlr4* gene expression in the CNS as a consequence of spinal cord injury that produces chronic pain. PCR analysis of spinal tissue following spinal L5 nerve transection revealed increased gene expression of *Tlr4* and the chaperone protein *Cd14* (Raghavendra, Tanga, & DeLeo, 2003; Tanga, Raghavendra, & DeLeo, 2004). The authors found mRNA expression of *Tlr4* and *Cd14* was rapidly induced at 4 hours post-injury with concomitant upregulation of the microglial activation marker integrin alpha M (*Itgam*, also known MAC-1 or CR3/CD11b), while astrocytic glial fibrillary acidic protein (*Gfap)* showed a delayed time-course of upregulation (Tanga, et al., 2004). This spinal injury-induced *Tlr4* upregulation was blocked by pre- or post-injury systemic administration of the drug minocycline, a putative glial modulator with non-selective pharmacological properties (Raghavendra, et al., 2003). Similar to L5 transection, increases in *Tlr4* and *Cd14* gene expression and TLR4 immunoreactivity in the rat spinal cord were also seen following an intraplantar injection of complete Freunds adjuvant, a model of peripheral inflammatory pain (Raghavendra, Tanga, & DeLeo, 2004; Zhao, Zhang, & Li, 2015). These correlative observations led to more definitive demonstrations of the involvement of TLR4 in a preclinical model of painful neuropathy. Mice harboring *Tlr4* mutations (i.e. the C3H/HeJ and C57BL/10ScCr strains) have attenuated mechanical hypersensitivity up to two weeks following spinal nerve transection, while rats given intrathecal treatment with TLR4 antisense oligonucleotide similarly reduced the behavioral hypersensitivity to spinal injury (Tanga, Nutile-McMenemy, & DeLeo, 2005). Markers of microglial activation and cytokine production were also suppressed by oligonucleotide antagonism of TLR4 (Tanga, et al., 2005), supporting a role for spinal microglia as contributing to TLR4-mediated neuropathic pain.

The generality of TLR4's involvement in neuropathic pain was confirmed with pharmacological interventions in an animal model of peripheral nerve injury, namely the chronic constriction injury (CCI) of the sciatic nerve. This partial nerve ligation produces robust mechanical allodynia in rats that is reversed when differing TLR4 antagonists are delivered intrathecally (Hutchinson, et al., 2008), ranging from LPS-RS (LPS from *Rhodobacter sphaeroides* with TLR4 antagonistic properties, although its mechanism of action may vary under different conditions (Döring, et al., 2017)) to the TLR4 antagonists (+)-naloxone and (+)-naltrexone (which do not bind the stereoselective opioid receptors (Wang, Zhang, et al., 2016)). Likewise, CCI-induced mechanical allodynia and thermal hyperalgesia in mice was reduced by systemic delivery of the TLR4 antagonist FP-1 (Bettoni, et al., 2008). Subcutaneous delivery of (+)-naloxone is also sufficient to acutely reverse spinal nerve ligation-induced allodynia in rats even when delivered months following injury (Lewis, et al., 2012), supporting TLR4 in the maintenance of ongoing pain. In agreement with these findings, intrathecal injection of TLR4 antagonist LPS-RS abrogates mechanical hypersensitivity in a mouse model of arthritic pain (Christianson, et al., 2011). While antagonism of TLR4 may help prevent dysregulated pain, engagement of TLR4 may

help orchestrate some aspects of tissue repair in the context of nerve injury (Boivin, et al., 2007). Indeed, intraspinal injection of the synthetic TLR4 agonist E6020 accelerates myelin debris clearance, Schwann cell infiltration, and remyelination in the rat spinal cord (Church, Milich, Lerch, Popovich, & McTigue, 2017). E6020 has lower affinity for TLR4 compared to LPS and shows less cross-reactivity with other TLRs, suggesting that proper tuning of TLR4 activation may lead to improved outcomes for demyelinating conditions with associated pain, such as spinal cord injury or multiple sclerosis.

Genetic perturbation of TLR4 provides further evidence for its role in nociceptive processing (Stokes, Cheung, Eddinger, Corr, & Yaksh, 2013; Stokes, Corr, & Yaksh, 2013). In wild-type mice that receive unilateral L5 nerve ligation, there is a rapid development of mechanical allodynia in the ipsilateral paw with minimal effects on contralateral paw withdrawal thresholds. In contrast, male mice lacking functional TLR4 (*Tlr4*$^{-/-}$) demonstrated partially attenuated mechanical hypersensitivity at later time points, which argues for the role of TLR4 in the transition from acute to chronic pain (Stokes, Cheung, et al., 2013). Similar results were obtained when employing the K/BxN serum transfer arthritis model of persistent pain; the mechanical sensitivity of *Tlr4*$^{-/-}$ mice gradually returned to baseline over weeks, while allodynia was maintained in wild-type mice (Christianson, et al., 2011). Furthermore, the absence of TLR4 reduces the severity of pain in an animal model of autoimmune neuropathy ($P0_{106–125}$-induced experimental autoimmune neuritis) (Brunn, et al., 2017). Rather than complete loss-of-function from gene knockout, short interfering RNA (siRNA) can be locally delivered to knockdown gene transcription. In this manner, intrathecal delivery of siRNA against TLR4 ameliorates CCI-induced neuropathic pain while suppressing IL-1β and TNF protein release in the spinal cord following CCI (Wu, et al., 2010).

In reflection of the anti-allodynic effects of TLR4 antagonism, intrathecal injection of the TLR4 agonist LPS produces allodynia in male (but not female) rats (Sorge, et al., 2011), a process driven by sexually dimorphic engagement of immune cells in the spinal cord (Sorge, et al., 2015), athough this finding is not without controversy (Costigan, Scholz, & Woolf, 2009; Krukowski, et al., 2016). A similar effect is observed in mice, where intrathecal LPS-evoked tactile allodynia was reduced in males by co-administration of the small molecule TLR4 antagonist TAK-242 while having no effect on females (Woller, et al., 2016). This leads to the intriguing possibility of sex differences regarding the involvement of innate immune signaling in the development of neuropathic tactile hypersensitivity. Given the predominance of chronic pain conditions disproportionately afflicting women, it has been argued that interactions between endocrine and immune mechanisms may help explain some of the sex differences observed in the epidemiology of pain disorders (Nicotra, et al., 2012; Rosen, Ham, & Mogil, 2017). These experiments underscore the importance of including males and females in both preclinical and clinical studies, and the generality of a proposed mechanism for pain if sex differences have not been considered.

There is general agreement that endogenous DAMPs recognized by TLR4 (see (Trotta, et al., 2014) are a likely culprit for TLR4-mediated pain, but it is unclear which DAMPs are essential in the process, as the nature of DAMP recognition depends upon tissue composition and could vary greatly depending on the nature of the pain-inducing injury.

Endogenous compounds that act as TLR4 ligands most likely act on various timescales, from those acutely released to changes in the extracellular matrix composition that occur during the protracted process of tissue repair or under conditions of chronic inflammation. However, several putative TLR4 ligands have been identified in the context of pain. HSP60 is a chaperone protein involved in mitochondrial protein folding, but under conditions of cellular stress or injury it can be released from cells (Ohashi, Burkart, Flohé, & Kolb, 2000). In primary culture of murine CNS cells, necrotic cells release HSP60, which results in a cycle of cell death by signaling at microglial TLR4 (Lehnardt, et al., 2008). An additional member of the heat shock protein family, HSP90, can potentiate TLR4 signaling and augments LPS-induced mechanical allodynia (Hutchinson, et al., 2009). Extracellular HMGB1 in the spinal cord has also been characterized as contributing to the development and maintenance of inflammatory pain in experimental arthritis based on its signaling through TLR4. Genetic or pharmacologic disruption of TLR4 blocked the pro-nociceptive effect of intrathecal HMGB1, which coincided with reduced spinal mRNA for glial activation and pro-inflammatory cytokine production (Agalave, et al., 2014). Only the partially oxidized disulfide HMGB1 isoform resulted in mechanical hypersensitivity (Agalave, et al., 2014), confirming that post-translational modifications of the redox state of HMGB1 influences its affinity for TLR4 or other receptors (Yang, Antoine, Andersson, & Tracey, 2013). Tenascin-C, an extracellular matrix glycoprotein, is lowly expressed at baseline but can be persistently upregulated during conditions of inflammation. In a model of arthritic joint disease known to induce pain, the fibrinogen-like globe domain of tenascin-C can stimulate the release of TNF in a manner that requires TLR4 but not CD14 or MD-2, implying the contribution of an alternative co-receptor (Midwood, et al., 2009). Another interesting candidate is galectin-3, which is expressed on microglia and released after CNS injury (Lalancette-Hébert, et al., 2012; Yip, et al., 2017) and LPS stimulation (Burguillos, et al., 2015). Galectin-3 is found in the DRG and dorsal horn of the spinal cord, and its inhibition is associated with decreased pro-inflammatory glial activation and concurrent pain attenuation in preclinical models of peripheral nerve injury and herpetic allodynia (Ma, Han, Wang, Ai, & Zheng, 2016; Takasaki, et al., 2012). This TLR4-dependent microglial release of galectin-3 can produce a self-renewing cycle of paracrine TLR4 action and galectin-3 release, which could partially explain the propagation of inflammatory loops even after the initial injury has been resolved (Burguillos, et al., 2015).

6. TLR2

TLR2 signaling

Toll-like receptor 2 (TLR2) resides at the cell surface is characterized by an exceptional diversity of exogenous and endogenous molecular patterns it recognizes, including a variety of lipid moieties (Aliprantis, et al., 1999; Brightbill, et al., 1999). This is due partly to its capacity for dimerizing with either TLR1 or TLR6, which expands the complexity of ligand specificity. Structural interrogation has confirmed TLR2 can distinguish a variety of lipopeptides by forming TLR2-TLR1 and TLR2-TLR6 heterodimers (Jin, et al., 2007; Kang, et al., 2009). Ligand-induced heterodimerization of the extracellular domains brings the cytosolic C-terminal TIR domains into proximity, which is postulated to initiate intracellular signaling through the MyD88-dependent pathway. Further complexity for ligand

discrimination is conferred through interactions with co-factors such as CD36 and CD14 (Lee, et al., 2012). Although the endogenous DAMPs for TLR2 have not been definitively captured at this level of resolution, there is circumstantial evidence that TLR2 can bind to HMGB1, HSP60, biglycan, hyaluronan, and many others (Jiang, et al., 2005; Park, et al., 2004; Schaefer, et al., 2005; Vabulas, et al., 2001). It is important to consider that, for the above citations, these alarmins were not exclusively recognized by TLR2, as there appears to be substantial overlap between these endogenous ligands and their effects on TLR2 and TLR4. The capacity for directly ascribing a functional consequence exclusively to TLR2 depends on the methodology employed, but also emphasizes the substantial redundancy and crosstalk between TLRs and their downstream targets.

The expression of TLR2 in the peripheral and central nervous systems is primarily on microglia and other macrophages, although some TLR2 has been detected at low levels in astrocytes, oligodendrocytes, Schwann cells, fibroblasts, endothelial cells, and neurons (Bsibsi, et al., 2002; Fan, Frey, & Malik, 2003; Olson & Miller, 2004; Tang, et al., 2007). In the brain, RNA sequencing of purified cell populations shows *Tlr2* gene expression is highly enriched in microglia compared to other cell types in the cortex (Zhang, et al., 2014).

TLR2 and persistent pain

Several lines of preclinical evidence have converged in support of TLR2 as contributing to persistent pain through neuroinflammatory mechanisms at nociceptive signaling pathways. Acute intrathecal delivery of a TLR2 agonist (heat killed *Listeria monocytogenes*) induces lasting tactile allodynia, but this pain response was absent in mice lacking functional *Tlr2* (Stokes, Corr, et al., 2013). In mixed spinal cord glial cell culture from wild type mice, incubation with the supernatant of damaged sensory neurons produces numerous pro-nociceptive inflammatory genes, such as TNF, IL-1β, IL-6, and iNOS (Kim, et al., 2007). In contrast, glia isolated from $Tlr2^{-/-}$ mutants show no change in cytokine and iNOS gene expression in response to damaged sensory neurons (Kim, et al., 2007). The authors further demonstrated that *Tlr2*-deficient mice have attenuated nerve transection-induced mechanical allodynia and thermal hyperalgesia compared to normal mice, which is accompanied by reduced glial activation and inflammatory gene expression in the spinal cord (Kim, et al., 2007). Nerve transection injury itself leads to increased TLR2 immunoreactivity in the spinal cord accompanied by increased microglial NADPH oxidase, an enzyme that produces reactive oxygen species (Lim, Kim, & Lee, 2013). Beyond the spinal cord, the absence of *Tlr2* in mice, like *Tlr4*, reduces markers of inflammation at the sciatic nerve in experimental autoimmune neuritis (Brunn, et al., 2017).

Other authors have similarly found *Tlr2* knockout to partially attenuate mechanical allodynia and thermal hyperalgesia induced by nerve ligation, although markers of glial activity in the lumbar spinal cord were still elevated ipsilateral to the nerve injury, compared to the contralateral lumbar laminae (Shi, Zekki, & Zhang, 2011; Stokes, Cheung, et al., 2013). Differences in the severity of the peripheral injury (transection vs. ligation) and differing time points of glial assessment post-injury may explain the discrepancy in microglial and astrocyte activation in *Tlr2* knockouts, while other groups have reported a dissociation between nociceptive hypersensitivity and conventional markers of “glial activation”

(Ulmann, et al., 2008). Alternatively, infiltrating macrophages at the site of the peripheral injury may also participate in the pro-nociceptive inflammatory response, as macrophages at the site of the sciatic nerve injury overexpress TLR2 and show evidence of NF-κB activation (Shi, et al., 2011). Likewise, peripheral nerve injury results in macrophage infiltration at mouse DRGs along with upregulation of monocyte chemoattractant protein-1 (MCP-1, also known as CCL2), but these inflammatory responses were abrogated in *Tlr2*-knockout mice (Kim, et al., 2011). Further characterizing the contribution of peripheral immune cells in chronic pain is advantageous for identifying pain biomarkers, as these cells are more readily accessible compared to the CNS. This is supported by patient data, where peripheral blood mononuclear cells isolated from patients suffering from chronic pain produce an exaggerated IL-1β response to TLR2, TLR4, and TLR7 agonists compared to pain-free controls (Kwok, Hutchinson, Gentgall, & Rolan, 2012). Taken together, these results suggest that TLR2 engagement following nerve injury promotes conditions of neuropathic pain, which is likely driven by the inflammatory consequences of its activation.

7. TLR5

TLR5 signaling

Toll-like receptor 5 (TLR5) as a mammalian PRR is known primarily for its recognition of bacterial flagellin, a structural protein of both Gram-negative and Gram-positive bacteria that forms the whip-like filament of flagellum used for bacterial motility. In this regard, TLR5 is one of the few TLRs where the primary pathogen-associated molecular pattern is a protein. The crystal structure of zebrafish TLR5 reveals the D1 domain of flagellin as the sequence contributing to binding and signaling at the TLR5 ectodomain (Yoon, et al., 2012). Receptor dimerization leads to signaling through the adaptor protein MyD88, which as described earlier results in activation of NF-κB and increased transcription of pro-inflammatory cytokines (Gewirtz, Navas, Lyons, Godowski, & Madara, 2001; Hayashi, et al., 2001). While TLR5 expression has been reported for microglia and astrocytes, often from primary glial cultures (Bowman, Rasley, Tranguch, & Marriott, 2003; Olson & Miller, 2004), it is less clear the expression profile for glia in and around the spinal cord *in vivo*. However, both microglia and astrocytes cultured from rat spinal cord produce a robust TNF response to flagellin, implying some functional TLR5 is present under these conditions (Stokes, Corr, et al., 2013). Likewise, TLR5 protein and mRNA expression is observed in mouse primary DRG sensory neuron cell cultures (Goethals, et al., 2010). Deeper characterization by *in situ* hybridization and immunohistochemistry has revealed the presence of TLR5-expressing medium-to-large diameter myelinated Aβ-fibers in mouse DRGs; these sensory neurons were confirmed to innervate the hindpaw glabrous skin and project to the deep laminae of the dorsal horn of the spinal cord, a pattern consistent for low-threshold Aβ-fibers whose transition to dysregulated firing following injury may underpin mechanical allodynia (Xu, et al., 2015).

TLR5 and persistent pain

In comparison to the other cell-surface TLRs, TLR5 has received relatively little attention regarding its possible involvement in pain. However, a few articles have offered circuit-level and molecular insight into its potential role in different models of neuropathic pain.

Reminiscent of the results obtained from *Tlr4*$^{-/-}$ mice, mechanical allodynia due to L5 nerve ligation was attenuated in *Tlr5*$^{-/-}$ mice, suggesting that full genetic deletion of *Tlr5* influences pain processing (Stokes, Cheung, et al., 2013). Although it is not entirely clear what endogenous ligand might interact with TLR5 under physiological conditions, HMGB1 has emerged as a candidate DAMP. Purified HMGB1 elicits TLR5-mediated signaling under a variety of *in vitro* conditions, including NF-κB-dependent transcription of inflammatory effectors such as TNF, IL-8, and iNOS through the MyD88 signaling cascade (Das, et al., 2016). Importantly, *in vivo* injection of flagellin or HMGB1 into the hindpaw of rats produces acute allodynia, the latter of which could be abrogated with co-administration of the TLR5 antagonist TH1020 (Das, et al., 2016). Taken together, these data support the hypothesis that pain amplification can occur through endogenous recognition of extracellular HMGB1 through TLR5. While these initial insights have been promising, future work is necessary to determine if TLR5-HMGB1 interactions are involved in a more general sense in sensory processing across alternative pain models, or if its function is more narrowly engaged for allodynia. Moreover, it is unclear if glial or neuroimmune signaling contribute in TLR5-mediated pain states, as they do for other TLRs, or if DRGs are the primary cellular participant.

8. TLR3, TLR7, TLR9: Emerging roles of endosomal TLRs and persistent pain

The contributions of the endosomal TLRs to innate immunity have been extensively explored concerning their nucleic acid sensing within the cytoplasmic compartment, but little is known regarding their involvement in pain perception. However, there is some initial evidence to suggest that these TLRs could modulate pain through both shared and distinct molecular mechanisms. This was indirectly supported by the observation that DRGs express TLR3, TLR7, and TLR9 in culture, and delivering specific agonists for TLR3 (poly I:C), TLR7 (gardiquimod), and TLR9 (CpG oligodeoxynucleotide (ODN) 1826) each increase TRPV1 expression and functional activity of these sensory neurons, with concomitant increases in the release of pro-nociceptive prostaglandin E_2 (Qi, et al., 2011). In addition to DRGs, these endosomal-residing TLRs are thought to be expressed in varying degrees on microglia, astrocytes, oligodendrocytes, Schwann cells, fibroblasts, endothelial cells (Bsibsi, et al., 2002; Goethals, et al., 2010; Jack, et al., 2005; Lehmann, et al., 2012; Olson & Miller, 2004).

TLR3

TLR3 recognizes double-stranded RNA (Alexopoulou, Holt, Medzhitov, & Flavell, 2001) and is unique among TLRs in that its activity is MyD88-independent; that is, it signals through the TRIF pathway, resulting in the release of type I interferons via IRF3 and/or inflammatory cytokines via NF-κB (Kawai & Akira, 2010). Beyond the previous observations in cultured DRGs, there are few investigations into the relationship between TLR3 and pain. A recent investigation identified elevated TLR3 mRNA and protein in the rat spinal cord following nerve injury, along with increased activation of microglial autophagy in response to nerve injury or application of TLR3 agonists (Chen & Lu, 2017). In a separate model of chronic pancreatitis, intrathecal infusion of TLR3 antisense

oligodeoxynucleotide to knockdown TLR3 expression resulted in modest relief from mechanical allodynia (Qian, et al., 2011), suggesting TLR3 mechanisms participate under certain conditions of inflammatory pain. Activation of TLR3 may also serve to modulate neuropathic pain, as *Tlr3*-deficient mice show modest reductions in nerve injury-induced allodynia (Stokes, Cheung, et al., 2013). What endogenous ligand would interact with TLR3 in the context of ongoing pain remains unclear, but host mRNA can act as a TLR3 ligand (Karikó, Ni, Capodici, Lamphier, & Weissman, 2004).

TLR7

TLR7 recognizes single-stranded RNA as its primary ligand (Diebold, Kaisho, Hemmi, Akira, & Reis e Sousa, 2004) and within acidified endosomes signals through the MyD88-dependent pathway to release NF-κB-mediated inflammatory cytokines or IRF7-mediated transcription of type I IFNs. As with other TLRs, TLR7 expression has been noted in microglia and astrocytes but is also localized to primary sensory neurons, in particular TRPV1-expressing C-fiber DRGs (Liu, Xu, Park, Berta, & Ji, 2010). The cellular and molecular basis of TLR7 and its role in pain was identified through functional interaction between microRNAs (miRNA) and transient receptor potential ankyrin 1 (TRPA1) receptors on DRG neurons. As a single-stranded RNA, the endogenous miRNA lethal-7 (let-7) can function as a ligand for TLR7. When applied to DRGs, let-7b binds TLR7 and produces coupling to TRPA1 channels, resulting in a reliable increase of inward current that was abolished if the cells were deficient in *Tlr7* or *Trpa1* (Park, Xu, et al., 2014). This mechanism of miRNA-induced hyperexcitability of nociceptors was supported behaviorally, as intraplantar injection of let-7b produced nocifensive pain and allodynia that was dependent on *Tlr7, Trpa1,* and *Myd88* but not *Trpv1* (Park, Xu, et al., 2014). Thus, in a similar manner to TLR5, there is accumulating evidence that TLR-mediated signaling on neurons is functionally relevant for encoding sensory modalities such as pain.

TLR9

TLR9 is localized to the luminal surface of lysosomes and is activated by DNA with unmethylated CpG dinucleotides (Ahmad-Nejad, et al., 2002; Hemmi, et al., 2000). Similar to TLR7, TLR9 signals through MyD88 to activate the transcription factor NF-κB for the production of pro-inflammatory cytokines TNF and IL-1β, or IRF7 for the production of IFNs. While TLR9 has been strongly implicated in autoimmune diseases (Marshak-Rothstein, 2006), its role in pain has been mostly unexplored. As with TLR4, direct stimulation of TLR9 appears to exacerbate neuronal injury through excessive glial release of TNF and nitric oxide (Iliev, Stringaris, Nau, & Neumann, 2004). Blocking TLR9 activation with CpG ODN 2088 reduces inflammatory signaling and improves tumor-induced thermal hyperalgesia when delivered systemically (Qi, et al., 2011). Targeted inhibition of TLR9 by intrathecal delivery of the same compound reduces thermal sensitivity evoked by spinal cord injury, suggesting broader applicability across pain models (David, et al., 2013). While this anti-nociceptive behavioral effect was attributed to reduced inflammation, a subsequent mechanistic investigation determined that TLR9 antagonism with CpG ODN 2088 influences cultured spinal astrocytes and neurons uniquely, suppressing IL-6, CCL2, and CXCL1 cytokine and chemokine release from astrocytes while protecting neurons from kainic acid-induced excitotoxicity (Acioglu, et al., 2016). Evidence is lacking to identify

what endogenous ligands may utilize this pathway after peripheral nerve injury. While these data implicate TLR9 in regulating thermal pain, its involvement in peripheral neuropathic pain has not been characterized.

9. TLR adaptor and accessory proteins

Much of the pleiotropic outcomes of TLR signaling are related to the diversity of co-factors that are involved in ligand discrimination and that modify its downstream signaling. The many studies that have uncovered an association of these adaptor and accessory proteins with some aspect of nociception further implicates TLR signaling mechanisms as contributing to persistent pain, although it can be challenging to interpret which TLR or class of TLRs are involved. For instance, the accessory protein CD14 significantly enhances TLR4 recognition of LPS but is also the TLR2-TLR6 heterodimer and endosomal TLR3, TLR7, and TLR9 (Lee, et al., 2012). CD14 may also contribute to neuropathic pain. In CD14-deficient mice (*Cd14*$^{-/-}$ KO) that received L5 nerve transection, both mechanical allodynia and thermal hypersensitivity were reduced compared to wild-type injured mice (Cao, Tanga, & DeLeo, 2009). Plasma fibronectin, a DAMP that parenchymal microglia are exposed to following vascular damage, induces a robust cytokine response (CCL2 and CXCL1) in mouse cells in a manner that required CD14 (Janova, et al., 2016). Genetic deletion of *Cd14* similarly attenuated microglial activation and monocyte recruitment after *in vivo* CNS injection of fibronectin or following CNS trauma and ischemic stroke (Janova, et al., 2016), all of which indicates CD14 may grant TLR4 (or other TLRs) some of its capacity for DAMP recognition and initiation of inflammatory events following certain injuries. The adaptor protein MyD88 is essential for nearly all TLR-mediated signaling. CCI increases MyD88 in nociceptive pathways including DRG and dorsal horn, as well as downstream phosphorylated NF-κB, phosphorylated ERK, and IL-1β. Intrathecal delivery of MyD88 homodimerization inhibitory peptide was sufficient to attenuate both mechanical allodynia and thermal hyperalgesia (Liu, et al., 2017). Conditional deletion of *Myd88* in $Na_v1.8$-expressing small-fiber sensory neurons altered neuropathic and inflammatory pain, although basal pain and acute inflammatory pain were unaffected (Liu, Liu, et al., 2016). These data further substantiate a role for TLRs in persistent pain, both in neuronal and non-neuronal cells.

10. Role of TLRs in opioid analgesia and chemotherapy-induced pain

The expanding notion of TLRs as sensors of damage or foreign compounds (i.e xenobiotics) has encouraged researchers to consider the extent that TLRs may accommodate xenobiotic-associated molecular patterns (XAMPs); that is, molecular motifs of non-pathogenic, non-self origin. Morphine, for instance, like other opioids, signal at receptors utilized by endogenous compounds (e.g. at μ opioid receptors), but the chemical structure and signaling kinetics of clinically-relevant opioid drugs differ from their endogenous counterparts. Indeed, there is evidence that morphine and other opioids result in neuroinflammatory responses that are mediated in part through glial expression of TLR4 (Grace, et al., 2016; Hutchinson, et al., 2010). Morphine binds to the hydrophobic core of MD-2 (similar to lipid A moiety from LPS) and induces TLR4 oligomerization, resulting in NF-κB-mediated release of IL-1β, TNF, and nitric oxide (Wang, et al., 2012). The implication of TLR

engagement in response to opioids is paradoxical hyperalgesia – that is, increased sensitivity to pain following opioid exposure. The mechanisms dictating morphine-induced nociceptive sensitization were recently described and include TLR4 and its signaling through NLRP3 inflammasomes in microglia (Grace, et al., 2016). This is of particular clinical interest, given the widespread use of prescription opioids for acute and chronic pain management.

Chemotherapy-induced peripheral neuropathy (CIPN) is a serious adverse outcome related to the cytotoxic effects of chemotherapy agents and is characterized by a constellation of symptoms, most commonly paresthesia, dysesthesia, allodynia, or hyperalgesia in the hands and feet that can persist long after the discontinuation of treatment. The precise mechanisms leading to CIPN are still an area of active investigation, but there is now evidence from several sources that some classes of chemotherapeutics can directly interface with TLR complexes, which could contribute in part to the painful outcomes of CIPN. Paclitaxel is widely-used chemotherapeutic that exhibits anti-cancer properties by stabilizing cellular microtubules against disassembly, but repeated administration leads to painful peripheral neuropathy (Polomano, Mannes, Clark, & Bennett, 2001). The observation that paclitaxel treatment resembles the pro-inflammatory response to LPS is supported by biochemical and structural evidence in murine and human cells that paclitaxel binds to MD2 and induces TLR4 oligomerization (Kawasaki, et al., 2000; Wang, et al., 2002; Zimmer, Liu, Clayton, Stephens, & Snyder, 2008). The ability for structurally dissimilar molecules such as opioids or paclitaxel to bind MD-2 confirms the notion that no structural similarity to LPS is required to interact with TLR4/MD-2 signaling complexes (Wang, Su, et al., 2016). Functionally, TLR4 in the DRG contributes to paclitaxel-induced peripheral neuropathy by sensitizing TRPV1+ neurons (Li, et al., 2015; Li, et al., 2014). Cisplatin, a platinum-based chemotherapy, is also clinically associated with CIPN, and the extent and duration of mechanical allodynia produced by cisplatin treatment was reduced in mice deficient in *Tlr3, Tlr4, Myd88,* and *Trif*, implying some partially overlapping yet indistinct inflammatory pathways contributing to allodynia (Park, Stokes, Corr, & Yaksh, 2014). These data implicate TLR complexes as a clear therapeutic target for CIPN, which could alleviate cancer morbidity and mortality that results from chemotherapy dose reduction or discontinuation in the face of unresolved pain.

11. Future directions and clinical implications

The prevalence of chronic pain is distressingly pervasive, with over 11% of adults in the United States reporting daily pain (Nahin, 2015). Our inability to properly manage pain is thought to be a major contributing factor to the dramatic rise in overdose deaths from prescription opioid drugs, leading to over 33,000 deaths per year and an economic burden of an estimated $78 billion (Florence, Zhou, Luo, & Xu, 2016; Rudd, Seth, David, & Scholl, 2016). These alarming statistics have led many to argue for need for research and investment in developing non-addictive analgesics (Grosser, Woolf, & FitzGerald, 2017). To this end, the cumulative evidence reviewed here supports the intriguing concept that TLR-mediated signaling on glia and neurons can powerfully influence pain signaling and could represent a novel therapeutic target for managing pain. However, there are several outstanding questions that warrant consideration.

First, when interpreting the preclinical evidence reviewed here, there are experimental caveats worth considering. Animals with mutant or knockout alleles for TLRs – using *Tlr4* as an example – are deficient in *Tlr4* across all cell types, which may have unrecognized consequences in biological systems not addressed by the investigators; that is, in these experiments it becomes difficult to attribute a genotype effect to resident or circulating immune cells, glia, neurons, alternative cells, or any combination therein. Moreover, not only have some TLR4 agonists been shown to signal around *Tlr4* null mutations compared to *Tlr4* knockouts (Goodridge, et al., 2007), but whole-body genetic deficiency in *Tlr4* could result in compensatory or off-target confounding effects during development (Rossi, et al., 2015), although the advent of cell type-specific and conditional knockout mice can address some of these experimental concerns. Pharmacological agents aimed at manipulating receptor activity avoid developmental complications of the genetic models, but issues of bioavailablity and penetrance across the blood-brain barrier are limiting factors (García Bueno, et al., 2016); moreover, questions of cell-type specificity usually cannot be addressed. Greater nuance is certainly needed for determining the cell type-specific localization and function of TLRs. Advanced cell sorting techniques have allowed immunologists to determine that the same TLR can have different signaling outcomes on different cells (Barbalat, et al., 2009), but what this means in the context of pain is largely unknown.

There are additional concerns regarding the purported effects of some drugs, which may not function purely as TLR antagonists. As an example, Döring, et al. (2017) recently discovered some agonistic effects of the presumptive antagonist LPS-RS when applied to murine microglia, both in culture and *in vivo.* This may call into question some of the data obtained that considered LPS-RS as a pure TLR4 receptor antagonist. While pharmacological and genetic techniques have still been extraordinarily informative, their limitations should still be considered when generating alternative explanations.

Second, despite excitement in the field regarding the contribution of TLRs to pain mechanisms, there are other PRRs not considered here that play essential roles in host immunity. There are several classes of PRRs that are localized in distinct cellular compartments, including those expressed at the plasma membrane (e.g. C-type lectin receptors (CLRs), dectins, and mannose receptors), those located at cytosolic membranes (e.g. nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs)), or those that function as soluble factors (e.g. pentraxins, collectins, ficolins, and complement receptors), collectively representing both extracellular and intracellular signal detection (Medzhitov, 2009). Dynamic and flexible regulatory networks allow these innate immune factors to cross-modulate each other in additive or subtractive ways (Cao, 2016). As an example, receptor for advanced glycation end-products (RAGE) is a PRR that shares overlapping features with many TLRs, such as its capacity for binding HMGB1, its signaling through p38 MAPK and JUN N-terminal kinase (JNK), and the resulting NF-κB-mediated transcription of pro-inflammatory immune molecules and oxidative species. Based on these similarities, others have implicated RAGE specifically, and PRRs more generally, as pathways for testing novel therapeutics against persistent pain (Allette, et al., 2014; Kato, et al., 2016).

Third, additional research is needed to more definitively identify the endogenous ligands that are presumed to activate TLRs under conditions of painful injury. Because the crystal structures of TLR-DAMP complexes have not been reported, a significant question remaining is how individual DAMPs interact with TLR signaling complexes. Using TLR4 as an example, the reviewed DAMPs are assumed to activate TLR4/MD-2 in a manner reminiscent of LPS, although further structural data is needed to confirm this prediction. In addition, the degree to which TLRs may directly bind DAMPs, or whether TLR signaling is engaged through indirect facilitation of cellular damage is not entirely clear, as researchers are often limited in their ability to dissociate causative versus circumstantial evidence for the involvement of TLRs. Elucidation of endogenous ligand recognition could eventually lead to improved pharmacotherapies by allowing for rational drug design based on structural data, rather than basing TLR treatments based on interactions with pathogenic molecular patterns.

Finally, a major challenge arises when hypothesizing therapeutic agents: which TLR do you target? Do you target them all? Based on the largely preclinical work examined here, broadly blocking TLR receptor function is a potential treatment option. However, TLRs are so widely expressed, so varying in the neurophysiological outcomes they support, and so important for initiating innate immune responses, broad receptor antagonism could have unintended negative consequences. Pharmacotherapies designed at directly antagonizing TLRs will need to address the concerns of immunosuppression and whether these drugs can be given after the emergence of symptomatic pain.

Further characterization of the DAMPs at the molecular, cellular, and systems level could lead to promising therapeutics targets that obviate the need for broad TLR antagonists. As an example, HMGB1 appears to be a shared ligand for the majority of TLRs covered here, and its extracellular release promotes hyperexcitability of peripheral sensory neurons leading to heightened states of pain (Feldman, Due, Ripsch, Khanna, & White, 2012). Likewise, many of the pro-inflammatory cytokines or immune mediators that are transcribed as a consequence of TLR engagement can contribute to sensory neuron hyperexcitability as well, such as MCP-1/CCL2 (White, et al., 2005). CCL2 or other inflammatory products could be targeted for resolution, although a clinical trial to inhibit CCR2, the cognate receptor of CCL2, reported only trends in alleviating paroxysmal pain and paresthesia/dysesthesia (Källiomaki, et al., 2013). While this methodology deserves further investigation, targeting individual immune mediators with small molecular inhibitors or neutralizing antibodies may be flawed, considering the list of molecules implicated in pain is vast and continues to grow. In this sense, developing methods for reducing transcription of the many pro-inflammatory mediators simultaneously could be a more effective approach. The positioning of MyD88 as a critical regulator for almost all TLR-mediated signaling makes it an attractive target for future investigation. Alternatively, utilizing anti-inflammatory cytokines as a means to resolve dysregulated inflammation during pain could also achieve this result. Such a treatment option has been tested in rodents, where gene therapy in the spinal cord to persistently upregulate expression of the anti-inflammatory cytokine IL-10 is successful at treating pain resulting from peripheral nerve injury, paclitaxel, and experimental autoimmune encephalomyelitis, a model of multiple sclerosis (Grace, et al., 2017; Ledeboer, et al., 2007; Milligan, et al., 2006). Similarly, a novel fusion protein of the anti-inflammatory cytokines IL-4 and IL-10 injected intrathecally reduces persistent inflammatory pain by

inhibiting the activity of glial cells and their inflammatory products (Eijkelkamp, et al., 2016). This method is additionally advantageous by avoiding the application of plasmid or viral vectors.

An intriguing recent report provides convincing evidence that LPS from Gram-negative bacteria in the gut can act at TLR4 on endothelial cells in the brain and induce cerebral cavernous malformations (a major cause of hemorrhagic stroke and seizure), but these TLR-induced malformations could be prevented by influencing the microbiome (Tang, et al., 2017). This surprising link between the composition of the gut and a serious brain disorder has been echoed in the pain literature as well, where the typical mechanical hypersensitivity to inflammatory pain is reduced in germ-free mice lacking intestinal microbiota (Amaral, et al., 2008). While speculative, manipulations to the composition of commensal microbiota could represent a strategy of influencing TLR function independent of typical pharmacological interventions.

12. Conclusion

Several decades of work have now clearly revealed that pain and inflammation are exquisitely entangled concepts. Here, we present evidence for Toll-like receptors as essential elements in the development and maintenance of persistent pain, although how each TLR contributes to pain does vary greatly based on its structure and cellular location. To this end, future research should seek to further dissect how each TLR contributes to nociception, and how its expression on glial cells or neurons differentially influences its effects on pain processing. The rapid progress made in unraveling the signaling properties of TLRs in the context of pain transduction has been utterly remarkable, but the difficulty faced in translating the preclinical insights into effective therapies can be disheartening. Despite these challenges, fundamental insights into how TLRs and innate immune signaling contribute to the pathogenesis of persistent pain have offered hope for a new frontier in pain management, one that moves beyond simply altering neuronal firing and considers targeting the neuroinflammatory phenotypes that contribute to chronic pain (Ji, Xu, & Gao, 2014). Continued elaboration of the role that each TLR plays in manifesting the pain experience is a promising opportunity for improving the treatment for the millions suffering each day in pain.

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Abbreviations

AP-1
activator protein-1

ATP
adenosine triphosphate

BDNF

brain-derived neurotrophic factor

CCI
chronic constriction injury

CCL2; MCP-1
chemokine C-C motif ligand 2; monocyte chemoattractant protein-1

CCL21
chemokine C-C motif ligand 21

CD14
cluster of differentiation 14

CIPN
chemotherapy-induced peripheral neuropathy

CNS
central nervous system

CSF-1
colony stimulating factor 1

CXCL1
chemokine C-X-C motif ligand 1

DAMP
damage-associated molecular pattern

DRG
dorsal root ganglia

GABA
γ-aminobutyric acid

GFAP
glial fibrillary acidic protein

HMGB1
high-mobility group box protein 1

HSP
heat shock protein

IFN
interferon

IL-1
interleukin-1

IL-1β
interleukin-1β

IL-1R
interleukin-1 receptor

IL-10
interleukin-10

IL-6
interleukin-6

IL-8
interleukin-8

iNOS
inducible nitric oxide synthase

IRF
IFN regulator factor

ITGAM; MAC-1; CR3/CD11b
integrin alpha M; macrophage-1 antigen; complement receptor 3/cluster of differentiation molecule 11B

L5
lumbar vertebral segment 5

LBP
LPS-binding protein

Let-7
lethal-7

LPS
lipopolysaccharide

LPS-RS
LPS from *Rhodobacter sphaeroides*

MAPK
mitogen-activated kinase

MD-2
myeloid differentiation factor 2

miRNA
microRNA

mRNA

messenger RNA

MyD88
myeloid differentiation primary response gene 88

NF-κB
nuclear factor-κB

ODN
oligodeoxynucleotide

PCR
polymerase chain reaction

PRR
pattern recognition receptor

RNA
ribonucleic acid

siRNA
short interfering RNA

TLR
Toll-like receptor

TNF
tumor necrosis factor

TRIF
Toll/interleukin-1 receptor (TIR)-domain-containing adaptor protein inducing interferon-β

TRPA1
transient receptor potential ankyrin 1

TRPV1
transient receptor potential vanilloid 1

References

Abbadie C, Lindia JA, Cumiskey AM, Peterson LB, Mudgett JS, Bayne EK, DeMartino JA, MacIntyre DE, Forrest MJ. Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. Proc Natl Acad Sci U S A. 2003; 100:7947–7952. [PubMed: 12808141]

Acioglu C, Mirabelli E, Baykal AT, Ni L, Ratnayake A, Heary RF, Elkabes S. Toll like receptor 9 antagonism modulates spinal cord neuronal function and survival: Direct versus astrocyte-mediated mechanisms. Brain Behav Immun. 2016; 56:310–324. [PubMed: 27044334]

Agalave NM, Larsson M, Abdelmoaty S, Su J, Baharpoor A, Lundbäck P, Palmblad K, Andersson U, Harris H, Svensson CI. Spinal HMGB1 induces TLR4-mediated long-lasting hypersensitivity and glial activation and regulates pain-like behavior in experimental arthritis. Pain. 2014; 155:1802–1813. [PubMed: 24954167]

Ahmad-Nejad P, Häcker H, Rutz M, Bauer S, Vabulas RM, Wagner H. Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. Eur J Immunol. 2002; 32:1958–1968. [PubMed: 12115616]

Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol. 2004; 4:499–511. [PubMed: 15229469]

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature. 2001; 413:732–738. [PubMed: 11607032]

Aliprantis AO, Yang RB, Mark MR, Suggett S, Devaux B, Radolf JD, Klimpel GR, Godowski P, Zychlinsky A. Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. Science. 1999; 285:736–739. [PubMed: 10426996]

Allette YM, Due MR, Wilson SM, Feldman P, Ripsch MS, Khanna R, White FA. Identification of a functional interaction of HMGB1 with Receptor for Advanced Glycation End-products in a model of neuropathic pain. Brain Behav Immun. 2014; 42:169–177. [PubMed: 25014009]

Amaral FA, Sachs D, Costa VV, Fagundes CT, Cisalpino D, Cunha TM, Ferreira SH, Cunha FQ, Silva TA, Nicoli JR, Vieira LQ, Souza DG, Teixeira MM. Commensal microbiota is fundamental for the development of inflammatory pain. Proc Natl Acad Sci U S A. 2008; 105:2193–2197. [PubMed: 18268332]

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. 1985; 42:791–798. [PubMed: 3931919]

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. 1985; 42:779–789. [PubMed: 3931918]

Arthur JS, Ley SC. Mitogen-activated protein kinases in innate immunity. Nat Rev Immunol. 2013; 13:679–692. [PubMed: 23954936]

Barbalat R, Lau L, Locksley RM, Barton GM. Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. Nat Immunol. 2009; 10:1200–1207. [PubMed: 19801985]

Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. Cell. 2009; 139:267–284. [PubMed: 19837031]

Batti L, Sundukova M, Murana E, Pimpinella S, De Castro Reis F, Pagani F, Wang H, Pellegrino E, Perlas E, Di Angelantonio S, Ragozzino D, Heppenstall PA. TMEM16F regulates spinal microglial function in neuropathic pain states. Cell Rep. 2016; 15:2608–2615. [PubMed: 27332874]

Bell JK, Botos I, Hall PR, Askins J, Shiloach J, Segal DM, Davies DR. The molecular structure of the Toll-like receptor 3 ligand-binding domain. Proc Natl Acad Sci U S A. 2005; 102:10976–10980. [PubMed: 16043704]

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; 56:1312–1319. [PubMed: 18615568]

Biber K, Tsuda M, Tozaki-Saitoh H, Tsukamoto K, Toyomitsu E, Masuda T, Boddeke H, Inoue K. Neuronal CCL21 up-regulates microglia P2X4 expression and initiates neuropathic pain development. EMBO J. 2011; 30:1864–1873. [PubMed: 21441897]

Boivin A, Pineau I, Barrette B, Filali M, Vallieres N, Rivest S, Lacroix S. Toll-like receptor signaling is critical for Wallerian degeneration and functional recovery after peripheral nerve injury. J Neurosci. 2007; 27:12565–12576. [PubMed: 18003835]

Botos I, Segal DM, Davies DR. The structural biology of Toll-like receptors. Structure. 2011; 19:447–459. [PubMed: 21481769]

Bowman CC, Rasley A, Tranguch SL, Marriott I. Cultured astrocytes express toll-like receptors for bacterial products. Glia. 2003; 43:281–291. [PubMed: 12898707]

Brightbill HD, Libraty DH, Krutzik SR, Yang RB, Belisle JT, Bleharski JR, Maitland M, Norgard MV, Plevy SE, Smale ST, Brennan PJ, Bloom BR, Godowski PJ, Modlin RL. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. Science. 1999; 285:732–736. [PubMed: 10426995]

Brunn A, Mihelcic M, Carstov M, Feind L, Wieser EC, Schmidt J, Utermohlen O, Deckert M. Toll-like receptor 2, Toll-like receptor 4, myeloid differentiation response gene 88, and Toll-IL-1 receptor domain-containing adaptor-inducing interferon-gamma (TRIF) selectively regulate susceptibility of P0106–125-induced murine experimental autoimmune neuritis. Am J Pathol. 2017; 187:42–54. [PubMed: 27842213]

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; 61:1013–1021. [PubMed: 12430718]

Buchanan MM, Hutchinson M, Watkins LR, Yin H. Toll-like receptor 4 in CNS pathologies. J Neurochem. 2010; 114:13–27. [PubMed: 20402965]

Burguillos MA, Svensson M, Schulte T, Boza-Serrano A, Garcia-Quintanilla A, Kavanagh E, Santiago M, Viceconte N, Oliva-Martin MJ, Osman AM, Salomonsson E, Amar L, Persson A, Blomgren K, Achour A, Englund E, Leffler H, Venero JL, Joseph B, Deierborg T. Microglia-secreted galectin-3 acts as a Toll-like receptor 4 ligand and contributes to microglial activation. Cell Rep. 2015; doi: 10.1016/j.celrep.2015.02.012

Calvo M, Zhu N, Tsantoulas C, Ma Z, Grist J, Loeb JA, Bennett DL. Neuregulin-ErbB signaling promotes microglial proliferation and chemotaxis contributing to microgliosis and pain after peripheral nerve injury. J Neurosci. 2010; 30:5437–5450. [PubMed: 20392965]

Cao L, Tanga FY, DeLeo JA. The contributing role of CD14 in toll-like receptor 4 dependent neuropathic pain. Neuroscience. 2009; 158:896–903. [PubMed: 18976692]

Cao X. Self-regulation and cross-regulation of pattern-recognition receptor signalling in health and disease. Nat Rev Immunol. 2016; 16:35–50. [PubMed: 26711677]

Chen GY, Nuñez G. Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol. 2010; 10:826–837. [PubMed: 21088683]

Chen W, Lu Z. Upregulated TLR3 promotes neuropathic pain by regulating autophagy in rat with L5 spinal nerve ligation model. Neurochem Res. 2017; 42:634–643. [PubMed: 28000161]

Chen Z, Muscoli C, Doyle T, Bryant L, Cuzzocrea S, Mollace V, Mastroianni R, Masini E, Salvemini D. NMDA-receptor activation and nitroxidative regulation of the glutamatergic pathway during nociceptive processing. Pain. 2010; 149:100–106. [PubMed: 20167432]

Choe J, Kelker MS, Wilson IA. Crystal structure of human toll-like receptor 3 (TLR3) ectodomain. Science. 2005; 309:581–585. [PubMed: 15961631]

Christianson CA, Dumlao DS, Stokes JA, Dennis EA, Svensson CI, Corr M, Yaksh TL. Spinal TLR4 mediates the transition to a persistent mechanical hypersensitivity after the resolution of inflammation in serum-transferred arthritis. Pain. 2011; 152:2881–2891. [PubMed: 22019135]

Church JS, Milich LM, Lerch JK, Popovich PG, McTigue DM. E6020, a synthetic TLR4 agonist, accelerates myelin debris clearance, Schwann cell infiltration, and remyelination in the rat spinal cord. Glia. 2017; 65:883–899. [PubMed: 28251686]

Costigan M, Scholz J, Woolf CJ. Neuropathic pain: a maladaptive response of the nervous system to damage. Annu Rev Neurosci. 2009; 32:1–32. [PubMed: 19400724]

Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature. 2005; 438:1017–1021. [PubMed: 16355225]

Das N, Dewan V, Grace PM, Gunn RJ, Tamura R, Tzarum N, Watkins LR, Wilson IA, Yin H. HMGB1 activates proinflammatory signaling via TLR5 leading to allodynia. Cell Rep. 2016; 17:1128–1140. [PubMed: 27760316]

David BT, Ratnayake A, Amarante MA, Reddy NP, Dong W, Sampath S, Heary RF, Elkabes S. A toll-like receptor 9 antagonist reduces pain hypersensitivity and the inflammatory response in spinal cord injury. Neurobiol Dis. 2013; 54:194–205. [PubMed: 23313320]

DeLeo JA, Tanga FY, Tawfik VL. Neuroimmune activation and neuroinflammation in chronic pain and opioid tolerance/hyperalgesia. Neuroscientist. 2004; 10:40–52. [PubMed: 14987447]

Didangelos A, Puglia M, Iberl M, Sanchez-Bellot C, Roschitzki B, Bradbury EJ. High-throughput proteomics reveal alarmins as amplifiers of tissue pathology and inflammation after spinal cord injury. Sci Rep. 2016; 6:21607. [PubMed: 26899371]

Diebold SS, 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; 303:1529–1531. [PubMed: 14976261]

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; 90:759–764. [PubMed: 21393555]

Döring C, Regen T, Gertig U, van Rossum D, Winkler A, Saiepour N, Bruck W, Hanisch UK, Janova H. A presumed antagonistic LPS identifies distinct functional organization of TLR4 in mouse microglia. Glia. 2017; 65:1176–1185. [PubMed: 28471051]

Eijkelkamp N, Steen-Louws C, Hartgring SA, Willemen HL, Prado J, Lafeber FP, Heijnen CJ, Hack CE, van Roon JA, Kavelaars A. IL4-10 fusion protein is a novel drug to treat persistent inflammatory pain. J Neurosci. 2016; 36:7353–7363. [PubMed: 27413147]

Fan J, Frey RS, Malik AB. TLR4 signaling induces TLR2 expression in endothelial cells via neutrophil NADPH oxidase. J Clin Invest. 2003; 112:1234–1243. [PubMed: 14561708]

Feldman P, Due MR, Ripsch MS, Khanna R, White FA. The persistent release of HMGB1 contributes to tactile hyperalgesia in a rodent model of neuropathic pain. J Neuroinflammation. 2012; 9:180. [PubMed: 22824385]

Florence CS, Zhou C, Luo F, Xu L. The economic burden of prescription opioid overdose, abuse, and dependence in the United States, 2013. Med Care. 2016; 54:901–906. [PubMed: 27623005]

García Bueno B, Caso JR, Madrigal JL, Leza JC. Innate immune receptor Toll-like receptor 4 signalling in neuropsychiatric diseases. Neurosci Biobehav Rev. 2016; 64:134–147. [PubMed: 26905767]

Gay NJ, Keith FJ. Drosophila Toll and IL-1 receptor. Nature. 1991; 351:355–356. [PubMed: 1851964]

Gay NJ, Symmons MF, Gangloff M, Bryant CE. Assembly and localization of Toll-like receptor signalling complexes. Nat Rev Immunol. 2014; 14:546–558. [PubMed: 25060580]

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; 167:1882–1885. [PubMed: 11489966]

Goethals S, Ydens E, Timmerman V, Janssens S. Toll-like receptor expression in the peripheral nerve. Glia. 2010; 58:1701–1709. [PubMed: 20578041]

Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. Nat Med. 2010; 16:1248–1257. [PubMed: 20948530]

Goodridge HS, McGuiness S, Houston KM, Egan CA, Al-Riyami L, Alcocer MJ, Harnett MM, Harnett W. Phosphorylcholine mimics the effects of ES-62 on macrophages and dendritic cells. Parasite Immunol. 2007; 29:127–137. [PubMed: 17266740]

Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. Nat Rev Immunol. 2014; 14:217–231. [PubMed: 24577438]

Grace PM, Loram LC, Christianson JP, Strand KA, Flyer-Adams JG, Penzkover KR, Forsayeth JR, van Dam AM, Mahoney MJ, Maier SF, Chavez RA, Watkins LR. Behavioral assessment of neuropathic pain, fatigue, and anxiety in experimental autoimmune encephalomyelitis (EAE) and attenuation by interleukin-10 gene therapy. Brain Behav Immun. 2017; 59:49–54. [PubMed: 27189037]

Grace PM, Strand KA, Galer EL, Urban DJ, Wang X, Baratta MV, Fabisiak TJ, Anderson ND, Cheng K, Greene LI, Berkelhammer D, Zhang Y, Ellis AL, Yin HH, Campeau S, Rice KC, Roth BL, Maier SF, Watkins LR. Morphine paradoxically prolongs neuropathic pain in rats by amplifying spinal NLRP3 inflammasome activation. Proc Natl Acad Sci U S A. 2016; 113:E3441–3450. [PubMed: 27247388]

Grosser T, Woolf CJ, FitzGerald GA. Time for nonaddictive relief of pain. Science. 2017; 355:1026–1027. [PubMed: 28280170]

Gu N, Peng J, Murugan M, Wang X, Eyo UB, Sun D, Ren Y, DiCicco-Bloom E, Young W, Dong H, Wu LJ. Spinal microgliosis due to resident microglial proliferation is required for pain hypersensitivity after peripheral nerve injury. Cell Rep. 2016; 16:605–614. [PubMed: 27373153]

Guan Z, Kuhn JA, Wang X, Colquitt B, Solorzano C, Vaman S, Guan AK, Evans-Reinsch Z, Braz J, Devor M, Abboud-Werner SL, Lanier LL, Lomvardas S, Basbaum AI. Injured sensory neuron-

derived CSF1 induces microglial proliferation and DAP12-dependent pain. Nat Neurosci. 2016; 19:94–101. [PubMed: 26642091]

Guo LH, Schluesener HJ. The innate immunity of the central nervous system in chronic pain: the role of Toll-like receptors. Cell Mol Life Sci. 2007; 64:1128–1136. [PubMed: 17440679]

Gwak YS, Hassler SE, Hulsebosch CE. Reactive oxygen species contribute to neuropathic pain and locomotor dysfunction via activation of CamKII in remote segments following spinal cord contusion injury in rats. Pain. 2013; 154:1699–1708. [PubMed: 23707296]

Hanamsagar R, Hanke ML, Kielian T. Toll-like receptor (TLR) and inflammasome actions in the central nervous system. Trends Immunol. 2012; 33:333–342. [PubMed: 22521509]

Hashimoto C, Hudson KL, Anderson KV. The Toll gene of Drosophila, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. Cell. 1988; 52:269–279. [PubMed: 2449285]

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; 410:1099–1103. [PubMed: 11323673]

Heiman A, Pallottie A, Heary RF, Elkabes S. Toll-like receptors in central nervous system injury and disease: a focus on the spinal cord. Brain Behav Immun. 2014; 42:232–245. [PubMed: 25063708]

Helley MP, Abate W, Jackson SK, Bennett JH, Thompson SW. The expression of Toll-like receptor 4, 7 and co-receptors in neurochemical sub-populations of rat trigeminal ganglion sensory neurons. Neuroscience. 2015; 310:686–698. [PubMed: 26434622]

Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S. A Toll-like receptor recognizes bacterial DNA. Nature. 2000; 408:740–745. [PubMed: 11130078]

Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K, Akira S. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. J Immunol. 1999; 162:3749–3752. [PubMed: 10201887]

Hutchinson MR, Ramos KM, Loram LC, Wieseler J, Sholar PW, Kearney JJ, Lewis MT, Crysdale NY, Zhang Y, Harrison JA, Maier SF, Rice KC, Watkins LR. Evidence for a role of heat shock protein-90 in toll like receptor 4 mediated pain enhancement in rats. Neuroscience. 2009; 164:1821–1832. [PubMed: 19788917]

Hutchinson MR, Zhang Y, Brown K, Coats BD, Shridhar M, Sholar PW, Patel SJ, Crysdale NY, Harrison JA, Maier SF, Rice KC, Watkins LR. Non-stereoselective reversal of neuropathic pain by naloxone and naltrexone: involvement of toll-like receptor 4 (TLR4). Eur J Neurosci. 2008; 28:20–29. [PubMed: 18662331]

Hutchinson MR, Zhang Y, Shridhar M, Evans JH, Buchanan MM, Zhao TX, Slivka PF, Coats BD, Rezvani N, Wieseler J, Hughes TS, Landgraf KE, Chan S, Fong S, Phipps S, Falke JJ, Leinwand LA, Maier SF, Yin H, Rice KC, Watkins LR. Evidence that opioids may have toll-like receptor 4 and MD-2 effects. Brain Behav Immun. 2010; 24:83–95. [PubMed: 19679181]

Iliev AI, Stringaris AK, Nau R, Neumann H. Neuronal injury mediated via stimulation of microglial toll-like receptor-9 (TLR9). FASEB J. 2004; 18:412–414. [PubMed: 14688201]

Jack CS, Arbour N, Manusow J, Montgrain V, Blain M, McCrea E, Shapiro A, Antel JP. TLR signaling tailors innate immune responses in human microglia and astrocytes. J Immunol. 2005; 175:4320–4330. [PubMed: 16177072]

Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002; 20:197–216. [PubMed: 11861602]

Janova H, Böttcher C, Holtman IR, Regen T, van Rossum D, Götz A, Ernst AS, Fritsche C, Gertig U, Saiepour N, Gronke K, Wrzos C, Ribes S, Rolfes S, Weinstein J, Ehrenreich H, Pukrop T, Kopatz J, Stadelmann C, Salinas-Riester G, Weber MS, Prinz M, Brück W, Eggen BJ, Boddeke HW, Priller J, Hanisch UK. CD14 is a key organizer of microglial responses to CNS infection and injury. Glia. 2016; 64:635–649. [PubMed: 26683584]

Ji RR, Chamessian A, Zhang YQ. Pain regulation by non-neuronal cells and inflammation. Science. 2016; 354:572–577. [PubMed: 27811267]

Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. Nat Rev Drug Discov. 2014; 13:533–548. [PubMed: 24948120]

Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y, Prestwich GD, Mascarenhas MM, Garg HG, Quinn DA, Homer RJ, Goldstein DR, Bucala R, Lee PJ, Medzhitov R, Noble PW. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nat Med. 2005; 11:1173–1179. [PubMed: 16244651]

Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, Lee H, Lee JO. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell. 2007; 130:1071–1082. [PubMed: 17889651]

Källiomaki J, Attal N, Jonzon B, Bach FW, Huizar K, Ratcliffe S, Eriksson B, Janecki M, Danilov A, Bouhassira D. AZD 2423 PTN Study Group. A randomized, double-blind, placebo-controlled trial of a chemokine receptor 2 (CCR2) antagonist in posttraumatic neuralgia. Pain. 2013; 154:761–767. [PubMed: 23523116]

Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, Han SH, Lee H, Paik SG, Lee JO. Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. Immunity. 2009; 31:873–884. [PubMed: 19931471]

Karikó K, Ni H, Capodici J, Lamphier M, Weissman D. mRNA is an endogenous ligand for Toll-like receptor 3. J Biol Chem. 2004; 279:12542–12550. [PubMed: 14729660]

Kato J, Agalave NM, Svensson CI. Pattern recognition receptors in chronic pain: Mechanisms and therapeutic implications. Eur J Pharmacol. 2016; 788:261–273. [PubMed: 27343378]

Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010; 11:373–384. [PubMed: 20404851]

Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity. 2011; 34:637–650. [PubMed: 21616434]

Kawasaki K, Akashi S, Shimazu R, Yoshida T, Miyake K, Nishijima M. Mouse toll-like receptor 4. MD-2 complex mediates lipopolysaccharide-mimetic signal transduction by Taxol. J Biol Chem. 2000; 275:2251–2254. [PubMed: 10644670]

Kawasaki Y, Zhang L, Cheng JK, Ji RR. Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J Neurosci. 2008; 28:5189–5194. [PubMed: 18480275]

Kim D, Kim MA, Cho IH, Kim MS, Lee S, Jo EK, Choi SY, Park K, Kim JS, Akira S, Na HS, Oh SB, Lee SJ. A critical role of toll-like receptor 2 in nerve injury-induced spinal cord glial cell activation and pain hypersensitivity. J Biol Chem. 2007; 282:14975–14983. [PubMed: 17355971]

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. [PubMed: 21951975]

Kronschläger MT, Drdla-Schutting R, Gassner M, Honsek SD, Teuchmann HL, Sandkühler J. Gliogenic LTP spreads widely in nociceptive pathways. Science. 2016; 354:1144–1148. [PubMed: 27934764]

Krukowski K, Eijkelkamp N, Laumet G, Hack CE, Li Y, Dougherty PM, Heijnen CJ, Kavelaars A. CD8+ T cells and endogenous IL-10 are required for resolution of chemotherapy-induced neuropathic pain. J Neurosci. 2016; 36:11074–11083. [PubMed: 27798187]

Kuner R. Central mechanisms of pathological pain. Nat Med. 2010; 16:1258–1266. [PubMed: 20948531]

Kwok YH, Hutchinson MR, Gentgall MG, Rolan PE. Increased responsiveness of peripheral blood mononuclear cells to in vitro TLR 2, 4 and 7 ligand stimulation in chronic pain patients. PLoS One. 2012; 7:e44232. [PubMed: 22937165]

Lacagnina MJ, Rivera PD, Bilbo SD. Glial and neuroimmune mechanisms as critical modulators of drug use and abuse. Neuropsychopharmacology. 2017; 42:156–177. [PubMed: 27402494]

Laflamme N, Rivest S. Toll-like receptor 4: the missing link of the cerebral innate immune response triggered by circulating gram-negative bacterial cell wall components. FASEB J. 2001; 15:155–163. [PubMed: 11149903]

Lalancette-Hébert M, Swarup V, Beaulieu JM, Bohacek I, Abdelhamid E, Weng YC, Sato S, Kriz J. Galectin-3 is required for resident microglia activation and proliferation in response to ischemic injury. J Neurosci. 2012; 32:10383–10395. [PubMed: 22836271]

Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain. 2009; 10:895–926. [PubMed: 19712899]

Ledeboer A, Jekich BM, Sloane EM, Mahoney JH, Langer SJ, Milligan ED, Martin D, Maier SF, Johnson KW, Leinwand LA, Chavez RA, Watkins LR. Intrathecal interleukin-10 gene therapy attenuates paclitaxel-induced mechanical allodynia and proinflammatory cytokine expression in dorsal root ganglia in rats. Brain Behav Immun. 2007; 21:686–698. [PubMed: 17174526]

Lee CC, Avalos AM, Ploegh HL. Accessory molecules for Toll-like receptors and their function. Nat Rev Immunol. 2012; 12:168–179. [PubMed: 22301850]

Lehmann SM, Kruger C, Park B, Derkow K, Rosenberger K, Baumgart J, Trimbuch T, Eom G, Hinz M, Kaul D, Habbel P, Kalin R, Franzoni E, Rybak A, Nguyen D, Veh R, Ninnemann O, Peters O, Nitsch R, Heppner FL, Golenbock D, Schott E, Ploegh HL, Wulczyn FG, Lehnardt S. An unconventional role for miRNA: let-7 activates Toll-like receptor 7 and causes neurodegeneration. Nat Neurosci. 2012; 15:827–835. [PubMed: 22610069]

Lehnardt S, Massillon L, Follett P, Jensen FE, Ratan R, Rosenberg PA, Volpe JJ, Vartanian T. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. Proc Natl Acad Sci U S A. 2003; 100:8514–8519. [PubMed: 12824464]

Lehnardt S, Schott E, Trimbuch T, Laubisch D, Krueger C, Wulczyn G, Nitsch R, Weber JR. A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. J Neurosci. 2008; 28:2320–2331. [PubMed: 18322079]

Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell. 1996; 86:973–983. [PubMed: 8808632]

Leulier F, Lemaitre B. Toll-like receptors--taking an evolutionary approach. Nat Rev Genet. 2008; 9:165–178. [PubMed: 18227810]

Lewis SS, Loram LC, Hutchinson MR, Li CM, Zhang Y, Maier SF, Huang Y, Rice KC, Watkins LR. (+)-naloxone, an opioid-inactive toll-like receptor 4 signaling inhibitor, reverses multiple models of chronic neuropathic pain in rats. J Pain. 2012; 13:498–506. [PubMed: 22520687]

Li Y, Adamek P, Zhang H, Tatsui CE, Rhines LD, Mrozkova P, Li Q, Kosturakis AK, Cassidy RM, Harrison DS, Cata JP, Sapire K, Zhang H, Kennamer-Chapman RM, Jawad AB, Ghetti A, Yan J, Palecek J, Dougherty PM. The cancer chemotherapeutic paclitaxel increases human and rodent sensory neuron responses to TRPV1 by activation of TLR4. J Neurosci. 2015; 35:13487–13500. [PubMed: 26424893]

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; 15:712–725. [PubMed: 24755282]

Lim H, Kim D, Lee SJ. Toll-like receptor 2 mediates peripheral nerve injury-induced NADPH oxidase 2 expression in spinal cord microglia. J Biol Chem. 2013; 288:7572–7579. [PubMed: 23386616]

Liu CC, Gao YJ, Luo H, Berta T, Xu ZZ, Ji RR, Tan PH. Interferon alpha inhibits spinal cord synaptic and nociceptive transmission via neuronal-glial interactions. Sci Rep. 2016; 6:34356. [PubMed: 27670299]

Liu F, Wang Z, Qiu Y, Wei M, Li C, Xie Y, Shen L, Huang Y, Ma C. Suppression of MyD88-dependent signaling alleviates neuropathic pain induced by peripheral nerve injury in the rat. J Neuroinflammation. 2017; 14:70. [PubMed: 28359290]

Liu T, Han Q, Chen G, Huang Y, Zhao LX, Berta T, Gao YJ, Ji RR. Toll-like receptor 4 contributes to chronic itch, alloknesis, and spinal astrocyte activation in male mice. Pain. 2016; 157:806–817. [PubMed: 26645545]

Liu T, Xu ZZ, Park CK, Berta T, Ji RR. Toll-like receptor 7 mediates pruritus. Nat Neurosci. 2010; 13:1460–1462. [PubMed: 21037581]

Liu XJ, Liu T, Chen G, Wang B, Yu XL, Yin C, Ji RR. TLR signaling adaptor protein MyD88 in primary sensory neurons contributes to persistent inflammatory and neuropathic pain and neuroinflammation. Sci Rep. 2016; 6:28188. [PubMed: 27312666]

Ma Z, Han Q, Wang X, Ai Z, Zheng Y. Galectin-3 inhibition is associated with neuropathic pain attenuation after peripheral nerve injury. PLoS One. 2016; 11:e0148792. [PubMed: 26872020]

Marshak-Rothstein A. Toll-like receptors in systemic autoimmune disease. Nat Rev Immunol. 2006; 6:823–835. [PubMed: 17063184]

Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol. 1994; 12:991–1045. [PubMed: 8011301]

Medzhitov R. Approaching the asymptote: 20 years later. Immunity. 2009; 30:766–775. [PubMed: 19538928]

Medzhitov R, Janeway C Jr. Innate immune recognition: mechanisms and pathways. Immunol Rev. 2000; 173:89–97. [PubMed: 10719670]

Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature. 1997; 388:394–397. [PubMed: 9237759]

Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, Janeway CA Jr. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol Cell. 1998; 2:253–258. [PubMed: 9734363]

Midwood K, Sacre S, Piccinini AM, Inglis J, Trebaul A, Chan E, Drexler S, Sofat N, Kashiwagi M, Orend G, Brennan F, Foxwell B. Tenascin-C is an endogenous activator of Toll-like receptor 4 that is essential for maintaining inflammation in arthritic joint disease. Nat Med. 2009; 15:774–780. [PubMed: 19561617]

Miller YI, Viriyakosol S, Binder CJ, Feramisco JR, Kirkland TN, Witztum JL. Minimally modified LDL binds to CD14, induces macrophage spreading via TLR4/MD-2, and inhibits phagocytosis of apoptotic cells. J Biol Chem. 2003; 278:1561–1568. [PubMed: 12424240]

Milligan ED, Sloane EM, Langer SJ, Hughes TS, Jekich BM, Frank MG, Mahoney JH, Levkoff LH, Maier SF, Cruz PE, Flotte TR, Johnson KW, Mahoney MM, Chavez RA, Leinwand LA, Watkins LR. Repeated intrathecal injections of plasmid DNA encoding interleukin-10 produce prolonged reversal of neuropathic pain. Pain. 2006; 126:294–308. [PubMed: 16949747]

Milligan ED, Watkins LR. Pathological and protective roles of glia in chronic pain. Nat Rev Neurosci. 2009; 10:23–36. [PubMed: 19096368]

Mogil JS. Animal models of pain: progress and challenges. Nat Rev Neurosci. 2009; 10:283–294. [PubMed: 19259101]

Nagyőszi P, Wilhelm I, Farkas AE, Fazakas C, Dung NT, Haskó J, Krizbai IA. Expression and regulation of toll-like receptors in cerebral endothelial cells. Neurochem Int. 2010; 57:556–564. [PubMed: 20637248]

Nahin RL. Estimates of pain prevalence and severity in adults: United States, 2012. J Pain. 2015; 16:769–780. [PubMed: 26028573]

Nicotra L, Loram LC, Watkins LR, Hutchinson MR. Toll-like receptors in chronic pain. Exp Neurol. 2012; 234:316–329. [PubMed: 22001158]

O'Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol. 2007; 7:353–364. [PubMed: 17457343]

O'Neill LA, Golenbock D, Bowie AG. The history of Toll-like receptors - redefining innate immunity. Nat Rev Immunol. 2013; 13:453–460. [PubMed: 23681101]

Ohashi K, Burkart V, Flohé S, Kolb H. Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. J Immunol. 2000; 164:558–561. [PubMed: 10623794]

Old EA, Clark AK, Malcangio M. The role of glia in the spinal cord in neuropathic and inflammatory pain. Handb Exp Pharmacol. 2015; 227:145–170. [PubMed: 25846618]

Olson JK, Miller SD. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol. 2004; 173:3916–3924. [PubMed: 15356140]

Ossipov MH, Dussor GO, Porreca F. Central modulation of pain. J Clin Invest. 2010; 120:3779–3787. [PubMed: 21041960]

Panter G, Jerala R. The ectodomain of the Toll-like receptor 4 prevents constitutive receptor activation. J Biol Chem. 2011; 286:23334–23344. [PubMed: 21543336]

Park BS, Song DH, Kim HM, Choi BS, Lee H, Lee JO. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. Nature. 2009; 458:1191–1195. [PubMed: 19252480]

Park CK, Xu ZZ, Berta T, Han Q, Chen G, Liu XJ, Ji RR. Extracellular microRNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. Neuron. 2014; 82:47–54. [PubMed: 24698267]

Park HJ, Stokes JA, Corr M, Yaksh TL. Toll-like receptor signaling regulates cisplatin-induced mechanical allodynia in mice. Cancer Chemother Pharmacol. 2014; 73:25–34. [PubMed: 24162377]

Park JS, Svetkauskaite D, He Q, Kim JY, Strassheim D, Ishizaka A, Abraham E. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J Biol Chem. 2004; 279:7370–7377. [PubMed: 14660645]

Peirs C, Seal RP. Neural circuits for pain: Recent advances and current views. Science. 2016; 354:578–584. [PubMed: 27811268]

Piccinini AM, Midwood KS. DAMPening inflammation by modulating TLR signalling. Mediators Inflamm. 2010

Polomano RC, Mannes AJ, Clark US, Bennett GJ. A painful peripheral neuropathy in the rat produced by the chemotherapeutic drug, paclitaxel. Pain. 2001; 94:293–304. [PubMed: 11731066]

Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science. 1998; 282:2085–2088. [PubMed: 9851930]

Qi J, Buzas K, Fan H, Cohen JI, Wang K, Mont E, Klinman D, Oppenheim JJ, Howard OM. Painful pathways induced by TLR stimulation of dorsal root ganglion neurons. J Immunol. 2011; 186:6417–6426. [PubMed: 21515789]

Qian NS, Liao YH, Feng QX, Tang Y, Dou KF, Tao KS. Spinal toll like receptor 3 is involved in chronic pancreatitis-induced mechanical allodynia of rat. Mol Pain. 2011; 7:15. [PubMed: 21342497]

Qureshi ST, Lariviere L, Leveque G, Clermont S, Moore KJ, Gros P, Malo D. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). J Exp Med. 1999; 189:615–625. [PubMed: 9989976]

Raghavendra V, Tanga F, DeLeo JA. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. J Pharmacol Exp Ther. 2003; 306:624–630. [PubMed: 12734393]

Raghavendra V, Tanga FY, DeLeo JA. Complete Freunds adjuvant-induced peripheral inflammation evokes glial activation and proinflammatory cytokine expression in the CNS. Eur J Neurosci. 2004; 20:467–473. [PubMed: 15233755]

Reeve AJ, Patel S, Fox A, Walker K, Urban L. Intrathecally administered endotoxin or cytokines produce allodynia, hyperalgesia and changes in spinal cord neuronal responses to nociceptive stimuli in the rat. Eur J Pain. 2000; 4:247–257. [PubMed: 10985868]

Roger T, Froidevaux C, Le Roy D, Reymond MK, Chanson AL, Mauri D, Burns K, Riederer BM, Akira S, Calandra T. Protection from lethal gram-negative bacterial sepsis by targeting Toll-like receptor 4. Proc Natl Acad Sci U S A. 2009; 106:2348–2352. [PubMed: 19181857]

Rosen S, Ham B, Mogil JS. Sex differences in neuroimmunity and pain. J Neurosci Res. 2017; 95:500–508. [PubMed: 27870397]

Rosetto M, Engstrom Y, Baldari CT, Telford JL, Hultmark D. Signals from the IL-1 receptor homolog, Toll, can activate an immune response in a Drosophila hemocyte cell line. Biochem Biophys Res Commun. 1995; 209:111–116. [PubMed: 7726823]

Rossi A, Kontarakis Z, Gerri C, Nolte H, Holper S, Kruger M, Stainier DY. Genetic compensation induced by deleterious mutations but not gene knockdowns. Nature. 2015; 524:230–233. [PubMed: 26168398]

Rudd RA, Seth P, David F, Scholl L. Increases in drug and opioid-involved overdose deaths - United States, 2010–2015. MMWR Morb Mortal Wkly Rep. 2016; 65:1445–1452. [PubMed: 28033313]

Schaefer L, Babelova A, Kiss E, Hausser HJ, Baliova M, Krzyzankova M, Marsche G, Young MF, Mihalik D, Gotte M, Malle E, Schaefer RM, Grone HJ. The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. J Clin Invest. 2005; 115:2223–2233. [PubMed: 16025156]

Schumann RR, Leong SR, Flaggs GW, Gray PW, Wright SD, Mathison JC, Tobias PS, Ulevitch RJ. Structure and function of lipopolysaccharide binding protein. Science. 1990; 249:1429–1431. [PubMed: 2402637]

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; 59:231–241. [PubMed: 21125644]

Shimazu R, Akashi S, Ogata H, Nagai Y, Fukudome K, Miyake K, Kimoto M. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J Exp Med. 1999; 189:1777–1782. [PubMed: 10359581]

Sorge RE, LaCroix-Fralish ML, Tuttle AH, Sotocinal SG, Austin JS, Ritchie J, Chanda ML, Graham AC, Topham L, Beggs S, Salter MW, Mogil JS. Spinal cord Toll-like receptor 4 mediates inflammatory and neuropathic hypersensitivity in male but not female mice. J Neurosci. 2011; 31:15450–15454. [PubMed: 22031891]

Sorge RE, Mapplebeck JC, Rosen S, Beggs S, Taves S, Alexander JK, Martin LJ, Austin JS, Sotocinal SG, Chen D, Yang M, Shi XQ, Huang H, Pillon NJ, Bilan PJ, Tu Y, Klip A, Ji RR, Zhang J, Salter MW, Mogil JS. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci. 2015; 18:1081–1083. [PubMed: 26120961]

Stokes JA, Cheung J, Eddinger K, Corr M, Yaksh TL. Toll-like receptor signaling adapter proteins govern spread of neuropathic pain and recovery following nerve injury in male mice. J Neuroinflammation. 2013; 10:148. [PubMed: 24321498]

Stokes JA, Corr M, Yaksh TL. Spinal toll-like receptor signaling and nociceptive processing: regulatory balance between TIRAP and TRIF cascades mediated by TNF and IFNbeta. Pain. 2013; 154:733–742. [PubMed: 23489833]

Strain SM, Fesik SW, Armitage IM. Characterization of lipopolysaccharide from a heptoseless mutant of Escherichia coli by carbon 13 nuclear magnetic resonance. J Biol Chem. 1983; 258:2906–2910. [PubMed: 6338007]

Takaoka A, Yanai H, Kondo S, Duncan G, Negishi H, Mizutani T, Kano S, Honda K, Ohba Y, Mak TW, Taniguchi T. Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. Nature. 2005; 434:243–249. [PubMed: 15665823]

Takasaki I, Taniguchi K, Komatsu F, Sasaki A, Andoh T, Nojima H, Shiraki K, Hsu DK, Liu FT, Kato I, Hiraga K, Kuraishi Y. Contribution of spinal galectin-3 to acute herpetic allodynia in mice. Pain. 2012; 153:585–592. [PubMed: 22197693]

Tang AT, Choi JP, Kotzin JJ, Yang Y, Hong CC, Hobson N, Girard R, Zeineddine HA, Lightle R, Moore T, Cao Y, Shenkar R, Chen M, Mericko P, Yang J, Li L, Tanes C, Kobuley D, Võsa U, Whitehead KJ, Li DY, Franke L, Hart B, Schwaninger M, Henao-Mejia J, Morrison L, Kim H, Awad IA, Zheng X, Kahn ML. Endothelial TLR4 and the microbiome drive cerebral cavernous malformations. Nature. 2017; 545:305–310. [PubMed: 28489816]

Tang SC, Arumugam TV, Xu X, Cheng A, Mughal MR, Jo DG, Lathia JD, Siler DA, Chigurupati S, Ouyang X, Magnus T, Camandola S, Mattson MP. Pivotal role for neuronal Toll-like receptors in ischemic brain injury and functional deficits. Proc Natl Acad Sci U S A. 2007; 104:13798–13803. [PubMed: 17693552]

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; 102:5856–5861. [PubMed: 15809417]

Tanga FY, Raghavendra V, DeLeo JA. Quantitative real-time RT-PCR assessment of spinal microglial and astrocytic activation markers in a rat model of neuropathic pain. Neurochem Int. 2004; 45:397–407. [PubMed: 15145554]

Tanji H, Ohto U, Shibata T, Miyake K, Shimizu T. Structural reorganization of the Toll-like receptor 8 dimer induced by agonistic ligands. Science. 2013; 339:1426–1429. [PubMed: 23520111]

Trotta T, Porro C, Calvello R, Panaro MA. Biological role of Toll-like receptor-4 in the brain. J Neuroimmunol. 2014; 268:1–12. [PubMed: 24529856]

Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, Inoue K. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature. 2003; 424:778–783. [PubMed: 12917686]

Ulmann L, Hatcher JP, Hughes JP, Chaumont S, Green PJ, Conquet F, Buell GN, Reeve AJ, Chessell IP, Rassendren F. Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve

injury mediates BDNF release and neuropathic pain. J Neurosci. 2008; 28:11263–11268. [PubMed: 18971468]

Vabulas RM, Ahmad-Nejad P, da Costa C, Miethke T, Kirschning CJ, Häcker H, Wagner H. Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. J Biol Chem. 2001; 276:31332–31339. [PubMed: 11402040]

Wadachi R, Hargreaves KM. Trigeminal nociceptors express TLR-4 and CD14: a mechanism for pain due to infection. J Dent Res. 2006; 85:49–53. [PubMed: 16373680]

Wall PD, Devor M. Sensory afferent impulses originate from dorsal root ganglia as well as from the periphery in normal and nerve injured rats. Pain. 1983; 17:321–339. [PubMed: 6664680]

Wang J, Kobayashi M, Han M, Choi S, Takano M, Hashino S, Tanaka J, Kondoh T, Kawamura K, Hosokawa M. MyD88 is involved in the signalling pathway for Taxol-induced apoptosis and TNF-alpha expression in human myelomonocytic cells. Br J Haematol. 2002; 118:638–645. [PubMed: 12139759]

Wang X, Loram LC, Ramos K, de Jesus AJ, Thomas J, Cheng K, Reddy A, Somogyi AA, Hutchinson MR, Watkins LR, Yin H. Morphine activates neuroinflammation in a manner parallel to endotoxin. Proc Natl Acad Sci U S A. 2012; 109:6325–6330. [PubMed: 22474354]

Wang X, Zhang Y, Peng Y, Hutchinson MR, Rice KC, Yin H, Watkins LR. Pharmacological characterization of the opioid inactive isomers (+)-naltrexone and (+)-naloxone as antagonists of toll-like receptor 4. Br J Pharmacol. 2016; 173:856–869. [PubMed: 26603732]

Wang Y, Su L, Morin MD, Jones BT, Whitby LR, Surakattula MM, Huang H, Shi H, Choi JH, Wang KW, Moresco EM, Berger M, Zhan X, Zhang H, Boger DL, Beutler B. TLR4/MD-2 activation by a synthetic agonist with no similarity to LPS. Proc Natl Acad Sci U S A. 2016; 113:E884–893. [PubMed: 26831104]

White FA, Sun J, Waters SM, Ma C, Ren D, Ripsch M, Steflik J, Cortright DN, Lamotte RH, Miller RJ. Excitatory monocyte chemoattractant protein-1 signaling is up-regulated in sensory neurons after chronic compression of the dorsal root ganglion. Proc Natl Acad Sci U S A. 2005; 102:14092–14097. [PubMed: 16174730]

Wiech K. Deconstructing the sensation of pain: The influence of cognitive processes on pain perception. Science. 2016; 354:584–587. [PubMed: 27811269]

Woller SA, Ravula SB, Tucci FC, Beaton G, Corr M, Isseroff RR, Soulika AM, Chigbrow M, Eddinger KA, Yaksh TL. Systemic TAK-242 prevents intrathecal LPS evoked hyperalgesia in male, but not female mice and prevents delayed allodynia following intraplantar formalin in both male and female mice: The role of TLR4 in the evolution of a persistent pain state. Brain Behav Immun. 2016; 56:271–280. [PubMed: 27044335]

Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science. 1990; 249:1431–1433. [PubMed: 1698311]

Wu FX, Bian JJ, Miao XR, Huang SD, Xu XW, Gong DJ, Sun YM, Lu ZJ, Yu WF. Intrathecal siRNA against Toll-like receptor 4 reduces nociception in a rat model of neuropathic pain. Int J Med Sci. 2010; 7:251–259. [PubMed: 20714435]

Xiang W, Chao ZY, Feng DY. Role of Toll-like receptor/MYD88 signaling in neurodegenerative diseases. Rev Neurosci. 2015; 26:407–414. [PubMed: 25870959]

Xu ZZ, Kim YH, Bang S, Zhang Y, Berta T, Wang F, Oh SB, Ji RR. Inhibition of mechanical allodynia in neuropathic pain by TLR5-mediated A-fiber blockade. Nat Med. 2015; 21:1326–1331. [PubMed: 26479925]

Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science. 2003; 301:640–643. [PubMed: 12855817]

Yan X, Weng HR. Endogenous interleukin-1beta in neuropathic rats enhances glutamate release from the primary afferents in the spinal dorsal horn through coupling with presynaptic N-methyl-D-aspartic acid receptors. J Biol Chem. 2013; 288:30544–30557. [PubMed: 24003233]

Yan X, Yadav R, Gao M, Weng HR. Interleukin-1 beta enhances endocytosis of glial glutamate transporters in the spinal dorsal horn through activating protein kinase C. Glia. 2014; 62:1093–1109. [PubMed: 24677092]

Yang H, Antoine DJ, Andersson U, Tracey KJ. The many faces of HMGB1: molecular structure-functional activity in inflammation, apoptosis, and chemotaxis. J Leukoc Biol. 2013; 93:865–873. [PubMed: 23446148]

Yao C, Oh JH, Lee DH, Bae JS, Jin CL, Park CH, Chung JH. Toll-like receptor family members in skin fibroblasts are functional and have a higher expression compared to skin keratinocytes. Int J Mol Med. 2015; 35:1443–1450. [PubMed: 25812726]

Yip PK, Carrillo-Jimenez A, King P, Vilalta A, Nomura K, Chau CC, Egerton AM, Liu ZH, Shetty AJ, Tremoleda JL, Davies M, Deierborg T, Priestley JV, Brown GC, Michael-Titus AT, Venero JL, Burguillos MA. Galectin-3 released in response to traumatic brain injury acts as an alarmin orchestrating brain immune response and promoting neurodegeneration. Sci Rep. 2017; 7:41689. [PubMed: 28128358]

Yoon SI, Kurnasov O, Natarajan V, Hong M, Gudkov AV, Osterman AL, Wilson IA. Structural basis of TLR5-flagellin recognition and signaling. Science. 2012; 335:859–864. [PubMed: 22344444]

Yowtak J, Lee KY, Kim HY, Wang J, Kim HK, Chung K, Chung JM. Reactive oxygen species contribute to neuropathic pain by reducing spinal GABA release. Pain. 2011; 152:844–852. [PubMed: 21296500]

Zanoni I, Ostuni R, Marek LR, Barresi S, Barbalat R, Barton GM, Granucci F, Kagan JC. CD14 controls the LPS-induced endocytosis of Toll-like receptor 4. Cell. 2011; 147:868–880. [PubMed: 22078883]

Zhang H, Nei H, Dougherty PM. A p38 mitogen-activated protein kinase-dependent mechanism of disinhibition in spinal synaptic transmission induced by tumor necrosis factor-alpha. J Neurosci. 2010; 30:12844–12855. [PubMed: 20861388]

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci. 2014; 34:11929–11947. [PubMed: 25186741]

Zhang YK, Liu JT, Peng ZW, Fan H, Yao AH, Cheng P, Liu L, Ju G, Kuang F. Different TLR4 expression and microglia/macrophage activation induced by hemorrhage in the rat spinal cord after compressive injury. J Neuroinflammation. 2013; 10:112. [PubMed: 24015844]

Zhao XH, Zhang T, Li YQ. The up-regulation of spinal Toll-like receptor 4 in rats with inflammatory pain induced by complete Freund's adjuvant. Brain Res Bull. 2015; 111:97–103. [PubMed: 25592618]

Zimmer SM, Liu J, Clayton JL, Stephens DS, Snyder JP. Paclitaxel binding to human and murine MD-2. J Biol Chem. 2008; 283:27916–27926. [PubMed: 18650420]

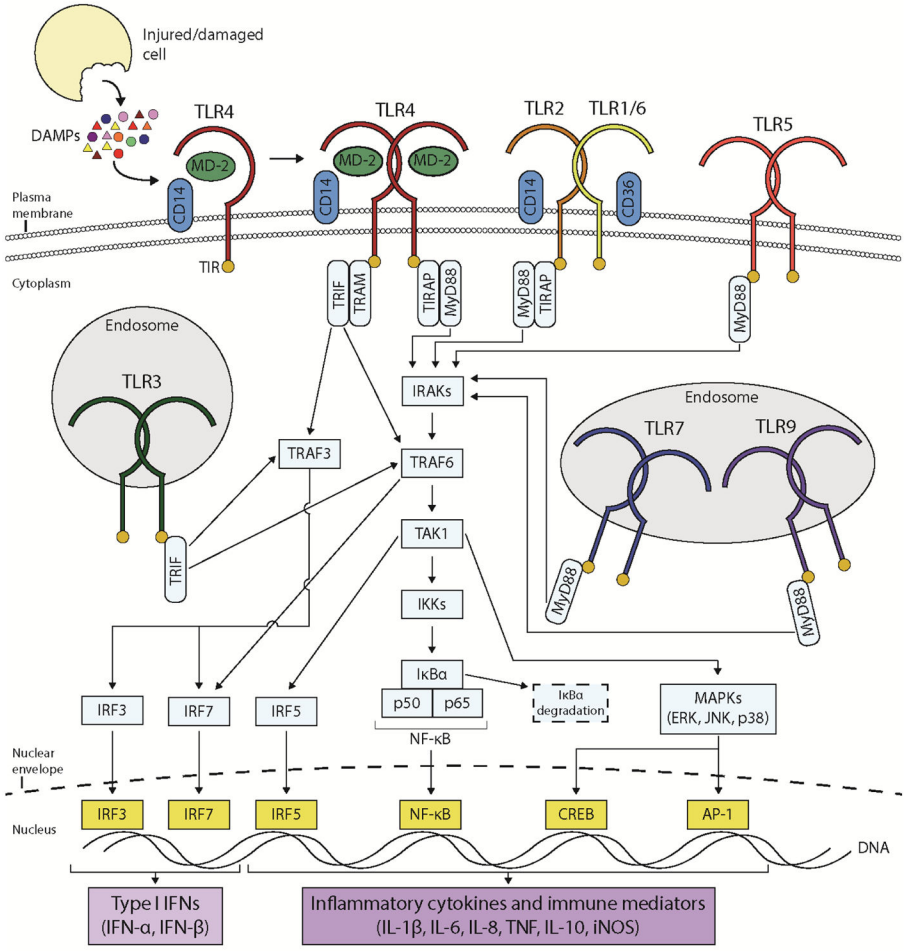

Fig. 1.

Table 1

Toll-like receptor	**TLR2/1/6**	**TLR3**	**TLR4**	**TLR5**	**TLR7/8**	**TLR9**
Subcellular localization	Cell surface	Endosome	Cell surface	Cell surface	Endosome	Endosome
Accessory proteins	CD14, CD36		CD14, MD-2			
Adaptor proteins	MyD88, TIRAP	TRIF	MyD88, TIRAP, TRIF, TRAM	MyD88	MyD88	MyD88
PAMP or exogenous ligand	Peptidoglycan Triacylated lipopeptides (with TLR1) Diacylated lipopeptides (with TLR6) Zymosan	dsRNA	LPS Lipid A	Flagellin	ssRNA Imidazoquinoline Gardiquimod Loxoribine	Unmethylated CpG DNA CpG ODN
DAMP	HSP60, HSP90 HMGB1 Hyaluronic acid Biglycan	mRNA	HSP60, HSP90 HMGB1 Tenascin-C Galectin-3 Fibronectin Hyaluronic acid Biglycan S100 proteins Oxidized lipoprotein	HMGB1	Self RNA	Self DNA HMGB1
Evidence of involvement in experimental models of pain	Nerve injury (L5 transection, SNL)[1] Autoimmune neuropathy[2] Receptor agonist injection[3]	Nerve injury (SNL)[1] Chronic pancreatitis[2]	Nerve injury (L5 transection, CCI, SNL)[1] Arthritic pain[2] Autoimmune neuropathy[3] Cancer pain[4] Chemotherapy- induced peripheral neuropathy (CIPN)[5] Opioid-induced hyperalgesia (OIH)[6] Receptor agonist injection[7]	Nerve injury (SNL)[1] Receptor agonist injection[2]	Receptor agonist injection[1] Bladder pain[2]	Spinal cord injury[1] Cancer pain[2]
References	[1](Kim, et al., 2007; Kim, et al., 2011; Shi, et al., 2011; Stokes, Cheung, et al., 2013) [2](Brunn, et al., 2017) [3](Stokes, Corr, et al., 2013)	[1](Stokes, Cheung, et al., 2013) [2](Qian, et al., 2011)	[1](Bettoni, et al., 2008; Hutchinson, et al., 2008; Stokes, Cheung, et al., 2013; Tanga, et al., 2005; Wu, et al., 2010) [2](Christianson, et al., 2011) [3](Brunn, et al., 2017) [4](Lan, et al., 2010) [5](Li, et al., 2015; Li, et al., 2014; Park, Stokes, et al., 2014; Shen, et al., 2017) [6](Grace, et al., 2016) [7](Sorge, et al., 2011; Woller, et al., 2016)	[1](Stokes, Cheung, et al., 2013) [2](Das, et al., 2016)	[1](Park, Xu, et al., 2014) [2](Ichihara, et al., 2017)	[1](David, et al., 2013) [2](Qi, et al., 2011)

Abbreviations: CCI, chronic constriction injury; CD, cluster of differentiation; CFA, complete Freund's adjuvant; DAMP, damage-associated molecular pattern; dsRNA, double-stranded RNA; HMGB1, high-mobility group box protein 1; HSP, heat shock protein; L5, lumbar vertebral segment 5; LPS, lipopolysaccharide; MD-2, myeloid differentiation factor 2; MyD88, myeloid differentiation primary response gene 88; ODN, oligodeoxynucleotide; PAMP, pathogen-associated molecular pattern; SNL, spinal nerve ligation; ssRNA, single-stranded RNA; TIRAP, TIR domain containing adaptor protein; TLR, Toll-like receptor; TRAM, TRIF-related adaptor molecule; TRIF, Toll/interleukin-1 (TIR)-domain-containing adaptor inducing interferon-β