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# Itch Control by Toll-Like Receptors

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## Abstract

Toll-like receptors (TLRs) are cellular sensors designed to recognize molecular danger signals associated with exogenous or endogenous threats. Their activation leads to initiation of the host's immune responses in order to remove or contain the danger. However, one of the most effective methods of defense against invading pathogens and parasites is itch. The perception of itch elicits the rapid defensive action to scratch, which can remove the offending pathogen or parasite before infection can occur. Recent findings show that TLRs such as TLR3, TLR4, and TLR7 are expressed in a subset of pruriceptive/nociceptive neurons in the dorsal root and trigeminal ganglion providing a direct link between TLR activation and itch. Activation of neuronal TLRs can initiate itch sensation by coupling with ion channels. Furthermore, TLRs are expressed in skin cells and glial cells in the spinal cord to regulate inflammation and neuroinflammation in chronic itch. Thus, identification of the role of TLRs in

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neurons, skin cells, and glial cells may provide new targets for the treatment of chronic itch, a common clinical problem associated with skin diseases, systemic diseases, and metabolic disorders, for which current treatments are far from sufficient.

### Keywords

Toll-like receptor • Itch • Pruritus • Glia • Dorsal horn • Dorsal root ganglia • Primary sensory neurons

## Abbreviations

5HT	Serotonin
BDNF	Brain-derived neurotrophic factor
CCL2	Chemokine ligand 2 (MCP-1)
CGRP	Calcitonin gene-related peptide
CNS	Central nervous system
COX-2	Cyclooxygenase 2
CXCL	Chemokine (C-X-C motif) ligand
DAMPs	Danger-associated molecular patterns
DRG	Dorsal root ganglia
dsRNA	Double-stranded RNA
ERK	Extracellular signal-regulated kinase
ET-1	Endothelin-1
GRP	Gastrin-releasing peptide
GRPR	Gastrin-releasing peptide receptor
IL-1 $\beta$	Interleukin-1beta
IRAKs	IL-1R-associated kinases
IRFs	Interferon regulatory factors
JNK	c-Jun N-terminal kinase
LPS	Lipopolysaccharide
LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein 1 (CCL2)
miRNA	Microribonucleic acid
mRNA	Messenger ribonucleic acid
NF-kB	Nuclear factor-kappa B
NGF	Nerve growth factor
NO	Nitric oxide
Nppb	Natriuretic polypeptide b
PAMPs	Pathogen-associated molecular patterns
PAR2	Protease-activated receptor 2
PGE2	Prostaglandin E2
Poly I:C	Polyinosinic:polycytidylic acid
RIP-1	Receptor-interacting protein 1

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sEPSCs	Spontaneous excitatory postsynaptic currents
ssRNA	Single-stranded RNA
TIR	Toll–interleukin-1 receptor
TLRs	Toll-like receptors
TNF- $\alpha$	Tumor necrosis factor alpha
TRAF6	Tumor necrosis factor receptor-associated factor 6
TRPV1	Transient receptor potential vanilloid subtype 1

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## 1 Introduction to Toll-Like Receptors

Toll-like receptors (TLRs) are pattern recognition receptors that are involved in the initiation of the innate immune response. Firstly, they recognize molecular patterns that are broadly shared by exogenous pathogens but that are distinguishable from host molecules. These are collectively referred to as pathogen-associated molecular patterns (PAMPs). Secondly, a subset of TLRs also recognize endogenous danger-associated molecular patterns (DAMPs) released in response to cellular stress or tissue injury. Thus, TLRs play a key role in initiating the immune responses to both foreign and endogenous threats.

When microbes were first recognized as the cause of infectious diseases, it was immediately clear that organisms must be capable of identifying when infected in order to initiate an immune response. Over a century ago, Richard Pfeiffer discovered “endotoxins” now known as lipopolysaccharide (LPS) which is produced by most Gram-negative bacteria (Westphal et al. 1978). Administration of LPS alone could provoke fever and shock in experimental animals. Additional molecules such as lipopeptides, flagellin, and unmethylated DNA could also provoke host immune responses; however, their receptors remained elusive.

The Toll receptor was first identified in *Drosophila melanogaster* and was reported for its role in dorsal–ventral patterning in the development (Anderson et al. 1985a, b). In 1996, Hoffmann and colleagues found that Toll also participated in the fly’s immune response to fungal infection (Lemaitre et al. 1996), and in 1997 Charles Janeway and Ruslan Medzhitov showed that activation of human Toll-like receptor 4 (TLR4) could promote an adaptive immune response (Medzhitov et al. 1997). Beutler and colleagues demonstrated that mice that could not respond to LPS had mutations that abolished the function of TLR4 and proved that TLR4 was in fact the receptor for LPS (Poltorak et al. 1998). Notably, Drs. Beutler and Hoffman were awarded the Nobel Prize in Medicine or Physiology for their pioneer work on TLRs in 2011. To date, 10 (TLR 1–10) and 12 (TLR 1–9; TLR 11–13) functional TLRs have been identified in human and mouse, respectively (Kawai and Akira 2010).

## 1.1 Structure

TLRs are type I transmembrane proteins comprised of an ectodomain responsible for detecting PAMPs or DAMPs, a transmembrane region consisting of a single  $\alpha$ -helix, and a cytosolic Toll–interleukin-1 receptor (TIR) domain that activates downstream signaling pathways (Kawai and Akira 2010). The extracellular domain binds either directly to ligands or to cofactors that can modulate their sensitivity to their natural ligand. In the case of TLR4, recognition of LPS requires the presence of MD-2, while cofactors CD14 and LPS-binding protein facilitate the presentation of LPS to MD-2. Upon stimulation, most TLRs form homodimers; however, some TLRs such as TLR1–TLR2 and TLR2–TLR6 also form heterodimers, each with a different ligand specificity (Triantafyllou et al. 2007; Oosting et al. 2011).

Structurally, TLRs may be classified into two groups. The first has structural transitions that divide the proteins into three subdomains: N terminal, central, and C terminal (Jin et al. 2007; Kim et al. 2007; Kang et al. 2009; Kang and Lee 2011). The ligand-binding pockets of TLR1, TLR2, and TLR6 are located close to the transition site between N terminal and central (Jin et al. 2007; Kang et al. 2009; Kang and Lee 2011), and the MD-2-binding pocket of TLR4 is located between the transition from central to C terminal (Kim et al. 2007). The second group has a single-domain configuration such as TLR3 (Choe et al. 2005; Bell et al. 2006). While the structures of TLR5, TLR7, TLR8, TLR9, and TLR10 have not yet been reported, sequence analysis suggests that the first four should belong to the single-domain group, while TLR10 should belong to the three-domain group (Kang and Lee 2011). These structural observations agree with functional analyses that have demonstrated that the three-domain TLRs interact with lipid-containing molecules such as LPS and lipoproteins. Accordingly, all three-domain TLRs (1, 2, 6, and 10) are expressed on the cell membrane for the detection of microbial membrane components. Conversely, the single-domain TLRs interact with hydrophilic ligands, such as viral or endogenous nucleic acids, and are expressed intracellularly on endosomes and the endoplasmic reticulum (TLR3, TLR7/TLR8, and TLR9). However, some TLRs (TLR3 and TLR7) are localized both on membrane and in intracellular compartments (Akira et al. 2006) (Table 1).

## 1.2 Ligands

Each type of TLR detects distinct molecular patterns specific to pathogenic threats to the organism such as viruses, bacteria, mycobacteria, fungi, and parasites. For example, TLR4 recognizes LPS (Poltorak et al. 1998; Shimazu et al. 1999), a component of Gram-negative bacterial cell membranes; TLR5 detects flagellin, a protein in bacterial flagella (Hayashi et al. 2001); and TLR11 senses profilin-like protein (Yarovinsky et al. 2005). TLR2 heterodimers bind to specific lipopeptides, another component of bacterial cell membranes. The ligand specificity of TLR2 is modulated by its heterodimeric partner. The TLR1–TLR2 complex binds to triacyl lipopeptides with only a weak affinity for diacyl lipopeptides, while the TLR2–

**Table 1** Subcellular localization and ligands of TLRs

TLR	Subcellular localization	Exogenous ligands	Origin of exogenous ligand	Endogenous ligands
TLR1/ TLR2	Cell surface	Triacyl lipopeptides (Pam3CSK4)	Bacteria, viruses	Unknown
		TLR2/ TLR6		
TLR3	Intracellular	dsRNA	Viruses	mRNA
	Cell surface	Poly I:C		Stathmin
TLR4	Cell surface	LPS	Bacteria, viruses	HSP-22, HSP-60, HSP-70
		Lipid A derivatives		HMGB1, fibronectin
				Defensin 2, oxLDL
				Tenascin C
TLR5	Cell surface	Flagellin	Bacteria	Unknown
TLR7	Intracellular	ssRNA Imidazoquinoline	Bacteria, viruses	Self-RNA
	Cell surface	Loxoribine		MicroRNA
		Bropirimine		
TLR8	Intracellular	ssRNA	Viruses	Self-RNA
		Imidazoquinoline		MicroRNA
TLR9	Intracellular	Unmethylated CpG DNA	Bacteria, viruses, protozoa	Self-DNA
		CpG ODNs		HMGB1
TLR10	Intracellular	Unknown	Unknown	Unknown
TLR11	Cell surface	Uropathogenic bacteria	Bacteria	Unknown
		Profilin-like molecules	Protozoa	

*CpG ODNs* CpG-containing oligodeoxynucleotides, *dsRNA* double-stranded RNA, *LPS* lipopolysaccharide, *LTA* lipoteichoic acid, n.d., *oxLDL* oxidized low-density lipoprotein, *PGN* peptidoglycan, *poly I:C* polyinosinic-polycytidylic acid, *ssRNA* single-stranded RNA

TLR6 complex interacts with diacyl lipopeptides but not to triacyl lipopeptides (Takeuchi et al. 2001, 2002; Alexopoulou et al. 2002; Yamamoto et al. 2002; Jin et al. 2007; Kang et al. 2009). TLR3 senses double-stranded RNAs and TLR7/TLR8 single-stranded RNAs, present in retroviruses and viruses, respectively (Alexopoulou et al. 2001; Diebold et al. 2004; Heil et al. 2004; Town et al. 2006). TLR9 senses unmethylated CpG DNA (Hemmi et al. 2000; Krieg 2002). Importantly, TLRs also recognize endogenous markers of cell necrosis and tissue injury, termed DAMPs. Activation of TLRs by DAMPs induces sterile

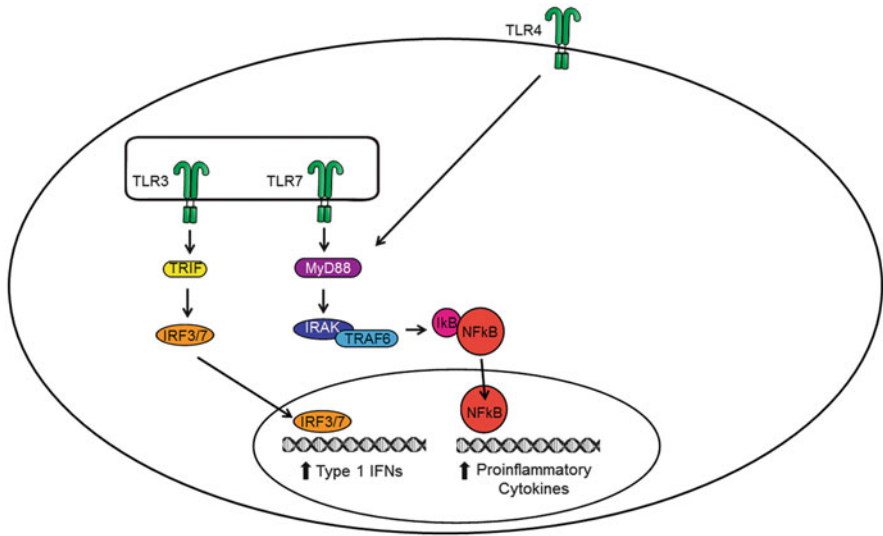
inflammatory responses (Okamura et al. 2001; Biragyn et al. 2002; Vabulas et al. 2002; Kariko et al. 2004; Jiang et al. 2005; Tian et al. 2007; Imai et al. 2008; Midwood et al. 2009; West et al. 2010). Thus, TLRs can recognize both pathogen invasion through their recognition of PAMPs and tissue injury through their recognition of endogenous DAMPs (summarized in Table 1).

### 1.3 Signaling Pathways

Activation of TLR signaling leads to a tightly regulated intracellular signaling pathway that initiates the production and secretion of proinflammatory mediators. The intracellular signaling domains of TLRs have a high sequence similarity with the interleukin-1 receptor and are termed Toll/IL-1R (TIR) homology domains. Signaling adaptor proteins MyD88, TRIF, and TRAM also contain TIR domains and interact with the TIR domains of the TLR receptors through heterotypic TIR–TIR interactions (Watters et al. 2007; Kenny and O’Neill 2008). TRIF and TRAM are also referred to as TICAM-1 and TICAM-2, respectively. All TLRs, with the exception of TLR3, signal through MyD88. TLR3 signals through a TRIF-dependent pathway discussed later. TLR4 is also capable of signaling in a MyD88-independent manner by recruiting TRAM (Yamamoto et al. 2003). TLR1/TLR2, TLR4, and TLR6 recruit the additional adapter protein TIRAP (Akira and Takeda 2004). Upon activation of the MyD88-dependent pathway, MyD88 recruits the IL-1R-associated kinases (IRAKs), which interact with tumor necrosis factor receptor-associated factor 6 (TRAF6), leading to the phosphorylation and degradation of the inhibitor of nuclear factor- $\kappa$ B (NF- $\kappa$ B) I $\kappa$ B. Degradation of I $\kappa$ B releases NF- $\kappa$ B which translocates to promote transcription of proinflammatory genes. The MyD88-dependent pathway also activates the mitogen-activated protein kinase (MAPK) signaling pathways, such as p38, and c-Jun N-terminal kinase (JNK), leading to the activation of AP-1 and interferon regulatory factors (IRFs) (see Fig. 1).

TLR3 signals through a TRIF-dependent pathway which activates two signaling pathways. First, TRIF recruits TRAF3 to activate IRF3 and IRF7 and initiate the production of type I interferons (e.g., IFN- $\alpha$ /IFN- $\beta$ ), which are the first line of defense produced by the innate immune system. Second, TRIF interacts with receptor-interacting protein 1 (RIP-1) and TRAF6 to activate NF- $\kappa$ B and/or MAPK pathways (Fig. 1). This is involved in the late-phase induction of proinflammatory genes.

The signaling molecules that comprise each TLR signaling pathway are also subject to a high degree of regulation through physical interactions, conformational changes, phosphorylation, ubiquitination, and proteasome-mediated degradation (Carpenter and O’Neill 2009). Some miRNAs are capable of regulating TLR signaling through targeting the 3’ untranslated regions of mRNAs encoding components of the TLR signaling system (O’Neill et al. 2011). Activation of TLR signaling produces a wide array of proinflammatory mediators, such as cytokines, chemokines, and reactive oxygen/nitrogen intermediates including nitric



**Fig. 1** Conventional signaling of TLRs. Activation of extracellular TLRs (e.g., TLR4) and intracellular TLRs (e.g., TLR3 and TLR7) initiates canonical TRIF- and MYD88-dependent signaling pathways resulting in the transcription of proinflammatory genes. Specifically, the activation of the transcriptional factors IRF3/IRF7 and NF- $\kappa$ B induces the production of inflammatory mediators such as cytokines (e.g., IL-1 $\beta$ , interferons), chemokines (e.g., CCL2), PGE<sub>2</sub> via the enzyme cyclooxygenase 2 (COX-2), and NO via the enzyme inducible nitric oxide synthase (iNOS). These inflammatory mediators act in concert to initiate the host's innate immune response

oxide (Takeda and Akira 2004). Upregulated activity of TLR signaling may promote chronic itch (Liu and Ji 2013).

## 2 Introduction to Itch

Itch, or pruritus, is defined as an unpleasant sensation that elicits the desire or reflex to scratch. While acute itch is an adaptive mechanism to warn an organism of potential chemical or parasitic danger (Ikoma et al. 2006), chronic itch is a common clinical problem associated with skin diseases (Reich and Szepietowski 2007; Bieber 2008), liver and kidney diseases (Cassano et al. 2010; Kremer et al. 2010), and metabolism disorders (Yamaoka et al. 2010). Additionally, itching itself leads to scratching which can cause inflammation, skin degradation, and secondary infection.

While some itch-sensing circuitry may overlap with existing nociceptive circuits, there are features that have recently been shown that are specific to itch. Itch sensation arises from MrgprA3<sup>+</sup> fibers, derived from a specific subset of primary sensory neurons that reside in the skin, but not in deeper tissues, muscles, organs, or bones where itch sensation does not occur (Han et al. 2013). Their cell bodies are located in the dorsal root ganglia (DRG) or trigeminal ganglia, while

their peripheral terminals, free nerve endings, reach to the stratum granulosum of the epidermis, and their central projections terminate in the superficial horn of the spinal cord. Itch-specific neurons terminate in lamina I–II and release glutamate as well as gastrin-releasing peptide (GRP), a neuropeptide known to elicit itch sensation via activation of GRP receptors (GRPRs) on superficial dorsal horn neurons (Sun and Chen 2007; Liu et al. 2009; Sun et al. 2009). A major source of GRP in the spinal cord may arise from interneurons (Alemi et al. 2013; Mishra and Hoon 2013). In addition, the neuropeptide natriuretic polypeptide b (Nppb) and substance P are also implicated in spinal cord itch transmission (Akiyama and Carstens 2013; Mishra and Hoon 2013). In the dorsal horn, itch signals can be processed and modulated before ascending to the brain where activation of specific brain regions then results in the perception of itch. Notably, in acute conditions, pain can suppress itch sensation via spinal cord inhibitory neurons (Liu et al. 2010b; Ross et al. 2010; Liu and Ji 2013).

The most characterized mediator of itch is histamine. The released histamine from local mast cells binds to H1/H4 receptors on nerve terminals (Shim and Oh 2008) which is followed by activation of PLCbeta3 and transient receptor potential vanilloid subtype 1 (TRPV1) (Han et al. 2006; Imamachi et al. 2009). TRPV1-positive C-fibers are required for both histamine-dependent and histamine-independent itch (Imamachi et al. 2009). Histamine-independent itch can be induced by the activation of transient receptor potential cation channel, subfamily A, member 1 (TRPA1), chloroquine (an antimalarial drug and MrgprA3 agonist), BAM8–22 (an endogenous MrgprC11 agonist), and oxidative stress (Liu et al. 2009; Wilson et al. 2011; Liu and Ji 2012), respectively.

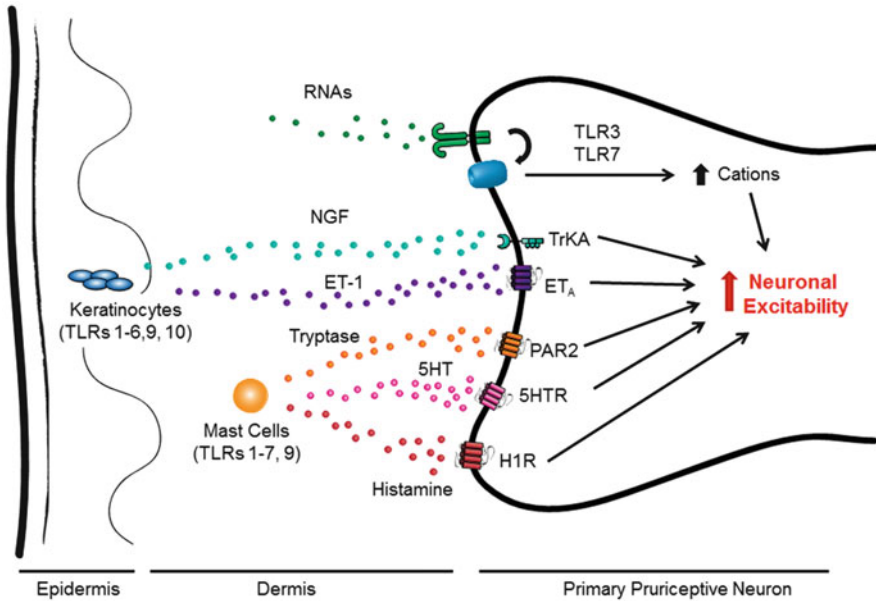
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### 3 Control of Itch by Peripheral TLRs

The skin is the body's largest organ and first line of defense against microbial and parasitic invaders. The skin expresses every type of known TLR receptor; however, each cell type has a unique expression pattern and distinct contribution to the skin's immune response (Ermertcan et al. 2011). In particular, two TLR-expressing cell type keratinocytes and mast cells have been implicated in chronic itch (Ikoma et al. 2006) (see Fig. 2).

Epidermal keratinocytes express TLRs 1–6, 9, and 10, which are upregulated in pruritic skin diseases, such as psoriasis and atopic dermatitis (Baker et al. 2003; Ermertcan et al. 2011). Mast cells, predominantly in the dermis, express TLRs 1–7 and 9 and play a key role in IgE-mediated allergic inflammation. Activation of these TLRs results in the synthesis and release of cytokines and chemokines to recruit immune cells from the circulation and mount an adaptive immune response. In particular, keratinocytes and mast cells are the major sources of nerve growth factor (NGF) (Ikoma et al. 2006). Intradermal administration of NGF enhances itch sensation in humans (Rukwied et al. 2013), and NGF is upregulated in dry skin models of itch in mice (Tominaga et al. 2007). Of interest, this increase is suppressed in *Tlr3* knockout animals (Liu et al. 2012). Therefore, TLR3-mediated





**Fig. 2** Involvement of peripherally expressed TLRs in itch sensation via different mechanisms. Cells residing in the skin such as keratinocytes and mast cells express TLRs. Their activation by exogenous ligands (PAMP) and endogenous ligands (DAMP) results in the release of multiple pruritogens including NGF, ET-1, 5HT, tryptase, and histamine, all of which can activate G protein coupled receptors (GPCRs) or TrkA receptor (in the case of NGF) on pruriceptive neuronal terminals to increase neuronal excitation and trigger itch sensation. TLRs such as TLR3 and TLR7 are also expressed by pruriceptor terminals, which can be activated by DAMP such as double-strand and single-strand RNAs. Activation of TLR3 and TLR7 elicits rapid inward currents and action potentials, due to the direct coupling with ion channels in pruriceptors, leading to increased neuronal excitation and itch sensation. A second late-phase response to the activation of TLRs may also involve the increased transcription of proinflammatory mediators which can maintain neuronal hyperexcitability in chronic itch conditions

release of NGF from skin cells may contribute to peripheral sensitization in chronic itch conditions.

While TLRs have mainly been studied on immune cells, we have recently demonstrated the expression of TLR3 and TLR7 on a subset of primary sensory neurons that are responsible for itch. Single-cell Real-time PCR (RT-PCR) and immunohistochemistry demonstrated that TLR7 is expressed in small TRPV1-expressing DRG neurons (Liu et al. 2010a, 2012) and is completely colocalized with MrgprA3<sup>+</sup>. TLR3 partially colocalizes with TLR7 and is also expressed in TRPV1-positive DRG neurons as shown by in situ hybridization, immunocytochemistry, single-cell RT-PCR, and electrophysiology (Liu et al. 2012).

TLR7 responds to synthetic ligands such as imidazoquinoline derivatives [e.g., imiquimod and resiquimod (R848)] and guanine analogs (e.g., loxoribine) (Hemmi et al. 2002). Intradermal injection of these synthetic compounds produces

scratching behavior in wild-type mice, which is reduced in *Tlr7*<sup>-/-</sup> mice, suggesting that these responses are, at least partially, TLR7 dependent (Liu et al. 2010a). *Tlr7*<sup>-/-</sup> mice also show a significant reduction of scratching behavior in response to non-histaminergic pruritogens such as chloroquine, endothelin-1 (ET-1), and SLIGRL-NH<sub>2</sub>, an agonist of protease-activated receptor 2 (PAR2) (Liu et al. 2010a). Interestingly, *Tlr7*<sup>-/-</sup> mice exhibited normal thermal and mechanical pain sensitivity. These findings show that TLR7 may serve an important role as a non-histaminergic itch receptor.

Activation of TLR7 in immune cells initiates signaling cascades leading to a variety of transcriptional changes to promote inflammation. However, in small DRG neurons, application of TLR7 agonists elicits rapid inward currents and action potentials, which are abolished in *Tlr7*<sup>-/-</sup> knockout mice (Liu et al. 2010a). This immediate increase in neuronal excitability suggests that TLR7 may be coupled to ion channel activation in primary sensory neurons. Our recent study shows that TLR7 is functionally coupled to TRPA1 but not TRPV1 (Park et al. 2014).

TLR3 responds to dsRNA as well as its synthetic analog, polyinosinic-polycytidylic acid (poly I:C), in which one strand of RNA is replaced by a polymer of inosinic acid. Activation of TLR3 by its ligands poly I:C or purified total RNAs also elicits rapid inward currents and the generation of action potentials from DRG neurons in wild-type but not *Tlr3*<sup>-/-</sup> mice (Liu et al. 2012). Furthermore, intradermal application of poly I:C produced dose-dependent scratching behavior in wild-type mice which was abolished in *Tlr3*<sup>-/-</sup> mice (Liu et al. 2012). Similar to TLR7, TLR3 also seems to serve as an itch receptor/co-receptor on pruriceptive neurons. In contrast to *Tlr7*<sup>-/-</sup> mice, which showed a partial reduction in histamine-independent itch, *Tlr3*<sup>-/-</sup> mice displayed significant reductions in both histamine-dependent and histamine-independent itch (Liu et al. 2012). Knockdown of TLR3 by intrathecal injection of *Tlr3* antisense oligonucleotides significantly reduced TLR3 expression in the DRG and reduced both histamine-dependent and histamine-independent itch in wild-type mice (Liu et al. 2012). This corroborates the results found using *Tlr3* knockout animals.

TLR3 and TLR7 expression by primary sensory neurons seems to serve as itch receptors to detect foreign pathogens and endogenous ligands (e.g., ds- and ssRNA, respectively), leading to a rapid defensive response: scratching. As neuronal excitation occurs within minutes of agonist application, the neuronal signaling pathway must have a distinct component from the traditional TLR signaling pathway in immune cells; however, the details remain to be determined. We postulate that TLR3 and TLR7 are expressed on the cell surface and are coupled to unidentified ion channels capable of inducing inward currents and action potentials (see Fig. 2). Of note, it was also demonstrated that the activation of TLRs, including TLR3, TLR7, and TLR9, in DRG neurons by their respective ligands may indirectly influence the excitability of DRG neurons by inducing the expression of proinflammatory mediators such as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), calcitonin gene-related peptide (CGRP), and interleukin-1beta (IL-1β) (Qi et al. 2011) (see Table 2). Thus, TLRs expressed by DRG neurons may regulate neuronal excitability by both transcriptional and non-transcriptional mechanisms.

**Table 2** Distribution of TLRs in the spinal cord and DRG

TLR	Cellular localization	Adapter proteins	Signaling pathway	Response
TLR2	Microglia	MyD88	NF-kB	TNF- $\alpha$ , IL-1 $\beta$ , BDNF, PGE2, NO
TLR3	Microglia	TRIF	p38	TNF- $\alpha$ , IL-1 $\beta$ , BDNF, PGE2, NO
	Astrocyte	TRIF	NF-kB JNK	IL-1 $\beta$ , CCL2, CXCL1, CXCL10
	DRG neurons	Unknown	ion channel coupling	Increased excitation
	Primary afferents	Unknown	ion channel coupling	Increased excitation
TLR4	Microglia	MyD88	NF-kB	TNF- $\alpha$ , IL-1 $\beta$ , BDNF, PGE2, NO
			ERK	
			p38	
	Astrocyte	MyD88	NF-kB ERK JNK	IL-1 $\beta$ , CCL2, CXCL1, CXCL10
TLR7	DRG neurons	MyD88	Ion channel coupling	Increased excitation

## 4 Control of Itch by Central TLRs

While both TLRs play a role in eliciting itch sensation from peripheral stimuli, only TLR3 was found to play a critical role in spinal synaptic transmission and the central sensitization underlying itch processing in the spinal cord (Liu et al. 2012). *Tlr3*<sup>-/-</sup> mice displayed decreased spontaneous excitatory postsynaptic currents (sEPSCs) in spinal lamina II neurons, while activation of TLR3 by poly I:C resulted in an increase in sEPSCs in wild-type animals (Liu et al. 2012). Furthermore, knockout of *Tlr3* abolished spinal long-term potentiation (LTP) following tetanic stimulation of the sciatic nerve (Liu et al. 2012). In contrast, both sEPSC activity and LTP remained intact in *Tlr7*<sup>-/-</sup> mice (Liu et al. 2012). Given the important role of spinal synaptic plasticity in itch hypersensitivity (Ross et al. 2010), impairments in central sensitization are likely to contribute to the profound itch deficit in *Tlr3*<sup>-/-</sup> mice.

Within the spinal cord, glial cells also express TLRs. Microglia, the resident immune cell of the central nervous system (CNS), express almost all known members of the TLR family, while astrocyte expression of TLRs is limited (Farina et al. 2007) (see Table 2). Similar to the activation of TLRs on peripheral immune cells, the activation of TLRs on glia results in the production and secretion of proinflammatory mediators including cytokines (e.g., TNF- $\alpha$ ), chemokines [e.g., chemokine ligand 2 (CCL2)], and enzymes [e.g., cyclooxygenase 2 (COX-2)], as well as other inflammatory mediators (e.g., prostaglandins) (Basbaum et al. 2009;

van Noort and Bsibsi 2009; Lehnardt 2010; Nicotra et al. 2011). While these mediators are known to generate central sensitization and pain hypersensitivity, their role in chronic itch is unknown.

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## 5 Clinical Significance and Future Perspectives

Chronic itch is a common and significant clinical problem. Chronic itch may involve the entire skin or be localized to a specific area or dermatome. It is more common in women than in men (Ständer et al. 2013), and its incidence increases with age (Weisshaar and Dalgard 2009; Ständer et al. 2010). It can be broadly categorized into four major etiologies: dermatologic causes (e.g., atopic eczema, psoriasis, or scabies), systemic causes (e.g., liver and kidney disease or metabolism disorders), neuropathic causes (such as spinal nerve impingement), and psychogenic causes (Yosipovitch and Bernhard 2013). However, regardless of the cause, it is associated with a marked reduction in the quality of life. In fact, a recent study showed that chronic itch was as debilitating as chronic pain (Kini et al. 2011). Clinically, the current treatments for chronic itch are far from sufficient (Yosipovitch and Bernhard 2013); however, targeting TLRs may offer new therapy options for treating debilitating itch-related problems.

TLRs are emerging as important players in the regulation of acute and chronic itch. They are expressed by many components of the itch signaling pathway including the cells of the skin, resident and infiltrating immune cells, peripheral and central neurons, as well as central glia. However, the precise contribution of TLR signaling in each cell type remains to be determined. Additionally, our work has raised the exciting possibility that TLR-mediated neuronal excitation occurs in a non-transcriptional manner. Again, much work remains to be done in order to elucidate the details of this pathway. These distinctions may provide targets for developing new therapies that block the detrimental effects of persistent unregulated TLR signaling, while leaving their beneficial effects intact.

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## References

- Akira S, Takeda K (2004) Toll-like receptor signalling. *Nat Rev Immunol* 4:499–511
- Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. *Cell* 124:783–801
- Akiyama T, Carstens E (2013) Neural processing of itch. *Neuroscience* 250:697–714
- Alemi F, Kwon E, Poole DP, Lieu T, Lyo V, Cattaruzza F, Cevikbas F, Steinhoff M, Nassini R, Materazzi S, Guerrero-Alba R, Valdez-Morales E, Cottrell GS, Schoonjans K, Geppetti P, Vanner SJ, Bunnett NW, Corvera CU (2013) The TGR5 receptor mediates bile acid-induced itch and analgesia. *J Clin Invest* 123:1513–1530

- Alexopoulou L, Holt AC, Medzhitov R, Flavell RA (2001) Recognition of double-stranded RNA and activation of NF- $\kappa$ B by Toll-like receptor 3. *Nature* 413:732–738
- Alexopoulou L, Thomas V, Schnare M, Lobet Y, Anguita J, Schoen RT, Medzhitov R, Fikrig E, Flavell RA (2002) Hyporesponsiveness to vaccination with *Borrelia burgdorferi* OspA in humans and in TLR1- and TLR2-deficient mice. *Nat Med* 8:878–884
- Anderson KV, Bokla L, Nusslein-Volhard C (1985a) Establishment of dorsal-ventral polarity in the *Drosophila* embryo: the induction of polarity by the Toll gene product. *Cell* 42:791–798
- Anderson KV, Jurgens G, Nusslein-Volhard C (1985b) Establishment of dorsal-ventral polarity in the *Drosophila* embryo: genetic studies on the role of the Toll gene product. *Cell* 42:779–789
- Baker BS, Ovigne JM, Powles AV, Corcoran S, Fry L (2003) Normal keratinocytes express Toll-like receptors (TLRs) 1, 2 and 5: modulation of TLR expression in chronic plaque psoriasis. *Br J Dermatol* 148:670–679
- Basbaum AI, Bautista DM, Scherrer G, Julius D (2009) Cellular and molecular mechanisms of pain. *Cell* 139:267–284
- Bell JK, Askins J, Hall PR, Davies DR, Segal DM (2006) The dsRNA binding site of human Toll-like receptor 3. *Proc Natl Acad Sci USA* 103:8792–8797
- Bieber T (2008) Atopic dermatitis. *N Engl J Med* 358:1483–1494
- Biragyn A, Ruffini PA, Leifer CA, Klyushnenkova E, Shakhov A, Chertov O, Shirakawa AK, Farber JM, Segal DM, Oppenheim JJ, Kwak LW (2002) Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. *Science* 298:1025–1029
- Carpenter S, O'Neill LA (2009) Recent insights into the structure of Toll-like receptors and post-translational modifications of their associated signalling proteins. *Biochem J* 422:1–10
- Cassano N, Tessari G, Vena GA, Girolomoni G (2010) Chronic pruritus in the absence of specific skin disease: an update on pathophysiology, diagnosis, and therapy. *Am J Clin Dermatol* 11:399–411
- Choe J, Kelker MS, Wilson IA (2005) Crystal structure of human toll-like receptor 3 (TLR3) ectodomain. *Science* 309:581–585
- Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C (2004) Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 303:1529–1531
- Ermercan AT, Ozturk F, Gunduz K (2011) Toll-like receptors and skin. *J Eur Acad Dermatol Venereol* 25:997–1006
- Farina C, Aloisi F, Meinl E (2007) Astrocytes are active players in cerebral innate immunity. *Trends Immunol* 28:138–145
- Han SK, Mancino V, Simon MI (2006) Phospholipase C $\beta$ 3 mediates the scratching response activated by the histamine H1 receptor on C-fiber nociceptive neurons. *Neuron* 52:691–703
- Han L, Ma C, Liu Q, Weng HJ, Cui Y, Tang Z, Kim Y, Nie H, Qu L, Patel KN, Li Z, McNeil B, He S, Guan Y, Xiao B, Lamotte RH, Dong X (2013) A subpopulation of nociceptors specifically linked to itch. *Nat Neurosci* 16:174–182
- Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410:1099–1103
- Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, Lipford G, Wagner H, Bauer S (2004) Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* 303:1526–1529
- Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S (2000) A Toll-like receptor recognizes bacterial DNA. *Nature* 408:740–745
- Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, Horiuchi T, Tomizawa H, Takeda K, Akira S (2002) Small anti-viral compounds activate immune cells via the TLR7/MyD88-dependent signaling pathway. *Nat Immunol* 3:196–200
- Ikoma A, Steinhoff M, Stander S, Yosipovitch G, Schmelz M (2006) The neurobiology of itch. *Nat Rev Neurosci* 7:535–547

- Imai Y, Kuba K, Neely GG, Yaghubian-Malhami R, Perkmann T, van Loo G, Ermolaeva M, Veldhuizen R, Leung YH, Wang H, Liu H, Sun Y, Pasparakis M, Kopf M, Mech C, Bavari S, Peiris JS, Slutsky AS, Akira S, Hultqvist M, Holmdahl R, Nicholls J, Jiang C, Binder CJ, Penninger JM (2008) Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. *Cell* 133:235–249
- Imamachi N, Park GH, Lee H, Anderson DJ, Simon MI, Basbaum AI, Han SK (2009) TRPV1-expressing primary afferents generate behavioral responses to pruritogens via multiple mechanisms. *Proc Natl Acad Sci USA* 106:11330–11335
- 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 (2005) Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat Med* 11:1173–1179
- Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, Lee H, Lee JO (2007) Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. *Cell* 130:1071–1082
- Kang JY, Lee JO (2011) Structural biology of the Toll-like receptor family. *Annu Rev Biochem* 80:917–941
- Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, Han SH, Lee H, Paik SG, Lee JO (2009) Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. *Immunity* 31:873–884
- Kariko K, Ni H, Capodici J, Lamphier M, Weissman D (2004) mRNA is an endogenous ligand for Toll-like receptor 3. *J Biol Chem* 279:12542–12550
- Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 11:373–384
- Kenny EF, O'Neill LA (2008) Signalling adaptors used by Toll-like receptors: an update. *Cytokine* 43:342–349
- Kim HM, Park BS, Kim JI, Kim SE, Lee J, Oh SC, Enkhbayar P, Matsushima N, Lee H, Yoo OJ, Lee JO (2007) Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist Eritoran. *Cell* 130:906–917
- Kini SP, DeLong LK, Veledar E, McKenzie-Brown AM, Schaufele M, Chen SC (2011) The impact of pruritus on quality of life: the skin equivalent of pain. *Arch Dermatol* 147:1153–1156
- Kremer AE, Martens JJ, Kulik W, Rueff F, Kuiper EM, van Buuren HR, van Erpecum KJ, Kondrackiene J, Prieto J, Rust C, Geenes VL, Williamson C, Moolenaar WH, Beuers U, Oude Elferink RP (2010) Lysophosphatidic acid is a potential mediator of cholestatic pruritus. *Gastroenterology* 139(1008–18):1018
- Krieg AM (2002) CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol* 20:709–760
- Lehnardt S (2010) Innate immunity and neuroinflammation in the CNS: the role of microglia in Toll-like receptor-mediated neuronal injury. *Glia* 58:253–263
- Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA (1996) The dorsoventral regulatory gene cassette *spatzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* 86:973–983
- Liu T, Ji RR (2012) Oxidative stress induces itch via activation of transient receptor potential subtype ankyrin 1 in mice. *Neurosci Bull* 28:145–154
- Liu T, Ji RR (2013) New insights into the mechanisms of itch: are pain and itch controlled by distinct mechanisms? *Pflugers Arch* 465:1671–1685
- Liu Q, Tang Z, Surdenikova L, Kim S, Patel KN, Kim A, Ru F, Guan Y, Weng HJ, Geng Y, Udem BJ, Kollarik M, Chen ZF, Anderson DJ, Dong X (2009) Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell* 139:1353–1365
- Liu T, Xu ZZ, Park CK, Berta T, Ji RR (2010a) Toll-like receptor 7 mediates pruritus. *Nat Neurosci* 13:1460–1462
- Liu Y, Abdel SO, Zhang L, Duan B, Tong Q, Lopes C, Ji RR, Lowell BB, Ma Q (2010b) VGLUT2-dependent glutamate release from nociceptors is required to sense pain and suppress itch. *Neuron* 68:543–556

- Liu T, Berta T, Xu ZZ, Park CK, Zhang L, Lu N, Liu Q, Liu Y, Gao YJ, Liu YC, Ma Q, Dong X, Ji RR (2012) TLR3 deficiency impairs spinal cord synaptic transmission, central sensitization, and pruritus in mice. *J Clin Invest* 122:2195–2207
- Medzhitov R, Preston-Hurlburt P, Janeway CA Jr (1997) A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 388:394–397
- Midwood K, Sacre S, Piccinini AM, Inglis J, Trebaul A, Chan E, Drexler S, Sofat N, Kashiwagi M, Orend G, Brennan F, Foxwell B (2009) Tenascin-C is an endogenous activator of Toll-like receptor 4 that is essential for maintaining inflammation in arthritic joint disease. *Nat Med* 15:774–780
- Mishra SK, Hoon MA (2013) The cells and circuitry for itch responses in mice. *Science* 340:968–971
- Nicotra L, Loram LC, Watkins LR, Hutchinson MR (2011) Toll-like receptors in chronic pain. *Exp Neurol* 234:316–329
- O'Neill LA, Sheedy FJ, McCoy CE (2011) MicroRNAs: the fine-tuners of Toll-like receptor signalling. *Nat Rev Immunol* 11:163–175
- Okamura Y, Watari M, Jerud ES, Young DW, Ishizaka ST, Rose J, Chow JC, Strauss JF III (2001) The extra domain A of fibronectin activates Toll-like receptor 4. *J Biol Chem* 276:10229–10233
- Oosting M, Ter HH, Sturm P, Adema GJ, Kullberg BJ, van der Meer JW, Netea MG, Joosten LA (2011) TLR1/TLR2 heterodimers play an important role in the recognition of *Borrelia* spirochetes. *PLoS One* 6:e25998
- Park CK, Xu ZZ, Berta T, Han QJ, Liu XJ, Ji RR (2014) Extracellular miRNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. *Neuron* 82:47–54
- 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 (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* 282:2085–2088
- Qi J, Buzas K, Fan H, Cohen JJ, Wang K, Mont E, Klinman D, Oppenheim JJ, Howard OM (2011) Painful pathways induced by TLR stimulation of dorsal root ganglion neurons. *J Immunol* 186:6417–6426
- Reich A, Szepletowski JC (2007) Mediators of pruritus in psoriasis. *Mediators Inflamm* 2007:1–6
- Ross SE, Mardinly AR, McCord AE, Zurawski J, Cohen S, Jung C, Hu L, Mok SI, Shah A, Savner EM, Toliás C, Corfas R, Chen S, Inquimbert P, Xu Y, McInnes RR, Rice FL, Corfas G, Ma Q, Woolf CJ, Greenberg ME (2010) Loss of inhibitory interneurons in the dorsal spinal cord and elevated itch in *Bhlhb5* mutant mice. *Neuron* 65:886–898
- Rukwied RR, Main M, Weinkauff B, Schmelz M (2013) NGF sensitizes nociceptors for cowhage-but not histamine-induced itch in human skin. *J Invest Dermatol* 133:268–270
- Shim WS, Oh U (2008) Histamine-induced itch and its relationship with pain. *Mol Pain* 4:29
- Shimazu R, Akashi S, Ogata H, Nagai Y, Fukudome K, Miyake K, Kimoto M (1999) MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med* 189:1777–1782
- Ständer S, Schafer I, Phan NQ, Blome C, Herberger K, Heigel H, Augustin M (2010) Prevalence of chronic pruritus in Germany: results of a cross-sectional study in a sample working population of 11,730. *Dermatology* 221:229–235
- Ständer S, Stumpf A, Osada N, Wilp S, Chatzigeorgakidis E, Pfliegerer B (2013) Gender differences in chronic pruritus: women present different morbidity, more scratch lesions and higher burden. *Br J Dermatol* 168:1273–1280
- Sun YG, Chen ZF (2007) A gastrin-releasing peptide receptor mediates the itch sensation in the spinal cord. *Nature* 448:700–703
- Sun YG, Zhao ZQ, Meng XL, Yin J, Liu XY, Chen ZF (2009) Cellular basis of itch sensation. *Science* 325:1531–1534
- Takeda K, Akira S (2004) TLR signaling pathways. *Semin Immunol* 16:3–9

- Takeuchi O, Kawai T, Muhlradt PF, Morr M, Radolf JD, Zychlinsky A, Takeda K, Akira S (2001) Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int Immunol* 13:933–940
- Takeuchi O, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z, Modlin RL, Akira S (2002) Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J Immunol* 169:10–14
- Tian J, Avalos AM, Mao SY, Chen B, Senthil K, Wu H, Parroche P, Drabic S, Golenbock D, Sirois C, Hua J, An LL, Audoly L, La RG, Bierhaus A, Naworth P, Marshak-Rothstein A, Crow MK, Fitzgerald KA, Latz E, Kiener PA, Coyle AJ (2007) Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat Immunol* 8:487–496
- Tominaga M, Ozawa S, Tengara S, Ogawa H, Takamori K (2007) Intraepidermal nerve fibers increase in dry skin of acetone-treated mice. *J Dermatol Sci* 48:103–111
- Town T, Jeng D, Alexopoulou L, Tan J, Flavell RA (2006) Microglia recognize double-stranded RNA via TLR3. *J Immunol* 176:3804–3812
- Triantafilou M, Uddin A, Maher S, Charalambous N, Hamm TS, Alsumaiti A, Triantafilou K (2007) Anthrax toxin evades Toll-like receptor recognition, whereas its cell wall components trigger activation via TLR2/6 heterodimers. *Cell Microbiol* 9:2880–2892
- Vabulas RM, Wagner H, Schild H (2002) Heat shock proteins as ligands of toll-like receptors. *Curr Top Microbiol Immunol* 270:169–184
- van Noort JM, Bsibi M (2009) Toll-like receptors in the CNS: implications for neurodegeneration and repair. *Prog Brain Res* 175:139–148
- Watters TM, Kenny EF, O’Neill LA (2007) Structure, function and regulation of the Toll/IL-1 receptor adaptor proteins. *Immunol Cell Biol* 85:411–419
- Weisshaar E, Dalgard F (2009) Epidemiology of itch: adding to the burden of skin morbidity. *Acta Derm Venereol* 89:339–350
- West XZ, Malinin NL, Merkulova AA, Tischenko M, Kerr BA, Borden EC, Podrez EA, Salomon RG, Byzova TV (2010) Oxidative stress induces angiogenesis by activating TLR2 with novel endogenous ligands. *Nature* 467:972–976
- Westphal O, Westphal U, Sommer T (1978) The history of pyrogen research. In: Schlessinger D (ed) *Microbiology*. American Society for Microbiology, Washington, DC, pp 221–238
- Wilson SR, Gerhold KA, Bifolck-Fisher A, Liu Q, Patel KN, Dong X, Bautista DM (2011) TRPA1 is required for histamine-independent, Mas-related G protein-coupled receptor-mediated itch. *Nat Neurosci* 14:595–602
- Yamamoto M, Sato S, Mori K, Hoshino K, Takeuchi O, Takeda K, Akira S (2002) Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-beta promoter in the Toll-like receptor signaling. *J Immunol* 169:6668–6672
- Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S (2003) Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science* 301:640–643
- Yamaoka H, Sasaki H, Yamasaki H, Ogawa K, Ohta T, Furuta H, Nishi M, Nanjo K (2010) Truncal pruritus of unknown origin may be a symptom of diabetic polyneuropathy. *Diabetes Care* 33:150–155
- Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, Hieny S, Sutterwala FS, Flavell RA, Ghosh S, Sher A (2005) TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science* 308:1626–1629
- Yosipovitch G, Bernhard JD (2013) Clinical practice. Chronic pruritus. *N Engl J Med* 368:1625–1634