



# Clinical Significance of Toll-Like Receptor and Toll-Like Receptor Blocker

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The mammalian Toll-like receptor (TLR) family, consisting of 13 members, plays an important role in innate recognition of specific patterns of microbial products. TLR-dependent recognition subsequently causes an activation of antigen-specific adaptive immunity. TLR-mediated signaling pathways consist of two pathways that induce gene expression: the myeloid differentiation primary response gene 88 (MyD88)-dependent pathway and Toll/interleukin-1 receptor-domain containing adaptor protein-inducing interferon- $\beta$ -dependent pathway. Synthetic TLR agonists, as well as TLR antagonists, affect and manipulate the host defense systems, and some of these immunomodulating agents may help to overcome intrinsic disturbances of the TLR system to offer new treatment options in urinary tract infection (UTI). Future studies are necessary to clarify additional associations between TLRs and severity of UTI, which may help in developing new treatment options.

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## INTRODUCTION

Toll-like receptors (TLRs) are a class of proteins that play an essential role in the innate immune system. They are single, membrane-spanning, non-catalytic receptors generally expressed in sentinel cells, including macrophages and dendritic cells (DCs), that recognize structurally conserved molecules derived from microbes. They received their name from their similarity to the protein coded by the toll gene recognized in *Drosophila* in 1985 by Christiane Nüsslein-Volhard. The researchers were so surprised that they spontaneously yelled out in German, “Das ist ja toll!” which translates as “That’s great!” [1]. The first mammalian homolog of the *Drosophila* Toll receptors was identified in 1997 as hToll (now termed TLR4) by Medzhitov et al. [2]. Subsequent studies have recognized several proteins that were structurally related to TLR4, and these were identified as TLRs.

TLRs include TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13, although the latter three have not been discovered in humans thus far [3].

## TLRs: MEDIATORS IN HOST IMMUNE RECOGNITION

TLRs, as components of the innate immune system, play a key role in the pathogenesis of urinary tract infection (UTI). They belong to the group of so-called pattern recognition receptors. TLRs sense and identify distinct pathogen-associated molecular patterns by homophilic and heterophilic interactions in order to defend mucosal barriers through the activation of immune cells and pro-inflammatory cytokines. TLRs interact with diverse endogenous and exogenous ligands. After binding—e.g., to lipopoly-

**Table 1.** Toll-like receptors and their ligands

TLR	Ligands
TLR1	Triacyl lipopeptides
TLR2	Peptidoglycan Lipopeptides, lipoteichoic acid, lipoarabinomannan, GPI anchors, phenol-soluble modulin, zymosan, glycolipids
TLR3	dsRNA
TLR4	LPS, Taxol, RSV fusion protein, MMTV envelope protein, endogenous ligand (HSPs, fibronectin, hyaluronic acid)
TLR5	Flagellin
TLR6	Diacyl lipopeptides
TLR7	ssRNA, imidazoquinolines
TLR8	ssRNA, imidazoquinolines (only in humans)
TLR9	CpG DNA
TLR10	Unknown
TLR11	Profilin, flagellin
TLR12	Profilin
TLR13	Bacterial 23S ribosomal RNA

TLR: Toll-like receptor, GPI: glycosylphosphatidylinositol, ds: double-stranded, LPS: lipopolysaccharide, RSV: respiratory syncytial virus, MMTV: mouse mammary tumor virus, HSPs: heat shock proteins, ss: single-stranded, CpG: cytosine-guanosine.

saccharide (LPS) (TLR4), peptidoglycans (TLR2), viral double-stranded (ds) RNA or DNA motifs (TLR3), extracellular matrix components, heat shock proteins (HSP), synthetic lipopeptides, and oligodeoxynucleotides—TLR signals produce an inflammatory response via the nuclear factor kappa-light-chain-enhancer of the activated B cells (NF- $\kappa$ B), secreting cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, and tumor necrosis factor-alpha (TNF- $\alpha$ ). As patient-related immunomodulating elements, TLRs are closely involved in pathogen identification and host defense in UTI [4,5]. Each TLR identifies a specific pattern of microbial parts (Table 1).

## 1. TLR1

TLR1 is functionally related to TLR2 [6]. An analysis of TLR1-deficient mice has shown the importance of TLR1 in the identification of triacyl lipopeptides [7]. Macrophages from TLR1 knockout (KO) mice demonstrated damaged inflammatory cytokine production in response to triacyl lipopeptides and lipoproteins from mycobacteria. Involvement of TLR1 in the identification of the outer surface lipoprotein of *Borrelia burgdorferi* has also been indicated. *B. burgdorferi* infections have been related to non-Hodgkins lymphoma [8].

## 2. TLR2

Several studies have indicated that TLR2 recognizes the components from a diverse array of microbial pathogens. These include lipoproteins from pathogens including Gram-

negative bacteria, *Mycoplasma fermentans*, *Treponema pallidum*, and *B. burgdorferi*, as well as peptidoglycan and lipoteichoic acid from Gram-positive bacteria, lipoarabinomannan from mycobacteria, glycosylphosphatidylinositol anchors from *Trypanosoma cruzi*, phenol-soluble modulin from *Staphylococcus epidermis*, zymosan from fungi, and glycolipids from *Treponema maltophilum*. The mechanism by which TLR2 identifies a wide diversity of microbial components is now described by the certainty that TLR2 cooperates with other TLRs, including TLR1 and TLR6, to make distinctions between specific patterns [9]. TLR2 expressed on tubular cells is actively participated in defending the urinary tract against uropathogenic *Escherichia coli* (UPEC). Stimulation of tubular cells in vitro by TLR2 ligands leads to the polarized secretion of inflammatory mediators, such as TNF- $\alpha$ , indicating that TLRs are also capable of combatting upper UTI [10].

## 3. TLR3

Alexopoulou et al. [11] found that TLR3 was involved in the recognition of dsRNA. dsRNA is produced by most viruses during replication. dsRNA induces the synthesis of type I interferons (IFN- $\alpha/\beta$ ), which apply antiviral and immunostimulatory activities, including the transcription of some IFN-inducible genes and maturation of DCs. Thus, TLR3 is implicated in the identification of dsRNA, thereby finding viral infection. TLR3 has been demonstrated to support cross-presentation of virus-infected cells through viral dsRNA-mediated activation of DCs [12].

#### 4. TLR4

LPS, a major component of the outer membrane of Gram-negative bacteria, shows potent immuno-stimulatory activity. Immoderate activation of monocytes and macrophages by LPS causes endotoxin shock, a systemic disease with a high mortality rate in humans and experimental animals. Therefore, the search for the LPS receptor has been ongoing. Hoshino et al. [13] found that TLR4 is vital for LPS signaling in TLR4 KO mice. TLR4 is implicated in the identification of several ligands in addition to LPS, including Taxol, mouse mammary tumor virus (MMTV) envelope protein, HSP, fibronectin, hyaluronic acid, and so on. Taxol is now utilized as an anti-tumor agent in clinical practice. In mice, Taxol has been demonstrated to possess many LPS-like activities, although the structure of Taxol is somewhat different from that of LPS [14,15]. In addition, the envelope glycoprotein of MMTV has been indicated to activate B cells by interacting with TLR4 [16]. Hence, TLR4 seems to be implicated in the identification of endogenous ligands borne on inflammatory response regardless of the source of infection.

TLR4 has clearly been shown to regulate the susceptibility to UTI, since TLR4 commands the earliest steps of the mucosal response towards UPEC. TLR4 activation in epithelial cells causes chemokine and cytokine production, while TLR4-KO mice improve an asymptomatic carrier state [17]. Chassin et al. [18] extended the previous data on a function of TLR4 expressed on innate immune cells and bladder epithelium by showing its importance in affecting UTI.

#### 5. TLR5

TLR5 has been identified as a specific TLR against bacterial flagellin [19]. Importantly, the expression of flagella in uropathogenic bacteria has previously been reported. Thus, TLR5 plays a critical, non-redundant function in the innate immune response in the urinary tract [20]. The association between TLR5 and the susceptibility to pneumonia caused by flagellated bacterium *Legionella pneumophila* has also been demonstrated [21].

#### 6. TLR6

As is the case for TLR1, TLR6 has been indicated to functionally be associated with TLR2. The introduction of dominant negative forms of TLR6 into the macrophage cell lines resulted in an inhibition of TNF- $\alpha$  production in

response to peptidoglycan, but not to bacterial lipopeptides, both of which are recognized by TLR2 [22]. An immunoprecipitation assay showed that TLR6 actually relates to TLR2. These results highlight the importance of TLR6 for the discrimination of TLR2 ligands [3].

#### 7. TLR7

TLR7 was first identified to be involved in the immune response to synthetic compounds approved for treatment of disorders related to viral infection. Imidazoquinolines were first identified as compounds with anti-viral activity in guinea pigs infected with herpes simplex virus, and are now utilized in clinical therapy of genital warts caused by infection of human papillomavirus. Imidazoquinolines generate the production of inflammatory cytokines, particularly IFN- $\alpha$ . TLR7 is responsible for imidazoquinoline-induced immune responses [23]. TLR7 has been shown to be related to human immunodeficiency virus, influenza virus, and autoimmune disorders, including systemic lupus erythematosus and Sjogren's syndrome [3,24,25].

#### 8. TLR8

TLR8 gene is extremely homologous to the TLR7 gene, and both genes are located on the X chromosome. Accordingly, human TLR8 recognizes the imidazoquinolines and single-stranded (ss) RNA, which are the ligands for TLR7. In contrast, mouse TLR8 does not identify such TLR7 ligands. This indicates that mouse TLR8 is probably nonfunctional [26].

#### 9. TLR9

TLR9 is important for the recognition of cytosine-guanosine (CpG) motif of bacterial and viral DNA. Similar to TLR7, TLR9 is related to the pathogenesis of autoimmune diseases [27]. Effective production of rheumatoid factor by autoreactive B cells has been indicated to be mediated by sequential joining of immunoglobulin G2a-chromatin complex with the B-cell receptor and TLR9 [27,28].

#### 10. TLR10

Human TLR10 has been recognized as a member with a close association with TLR1 and TLR6. Ligand of TLR10 remains unclear. TLR10 may be associated with the recognition of TLR2 ligands [3]. A mouse homolog of TLR10 has not yet been recognized.

## 11. TLR11

TLR11 is nonfunctional in humans, because a stop codon is inserted in the TLR11 gene. Expression of mouse TLR11 has been demonstrated in epithelial cells of the bladder and it intercedes with resistance to infection by uropathogenic bacteria. Mice that lack TLR11 are very susceptible to infection of the kidneys by UPEC, which suggests that TLR11 may play a key role in preventing infection of the urogenital organs [29]. In addition, TLR11 identifies flagellin of *Salmonella typhimurium* [30].

## 12. TLR12

TLR12, which is extremely homologous to TLR11, identifies profilin derived from *Toxoplasma gondii* [31]. The ligand for TLR12 is profilin.

## 13. TLR13

TLR13 identifies bacterial 23S ribosomal RNA [31,32]. The sequence of bacterial 23S ribosomal RNA found by TLR13 has been recognized as a conserved CGGAAAGACC motif [33].

## TLR SIGNALING PATHWAYS

Engagement of TLRs by microbial components brings out conformational changes in TLRs required for the recruitment of Toll/interleukin-1 receptor (TIR)-domain-containing adaptor molecules to the TIR domain of TLRs [9]. Moreover, dimerization of TLRs activates the signaling pathways originating from a cytoplasmic TIR domain. In the signaling pathways downstream of the TIR domain, a TIR domain-containing adaptor, myeloid differentiation primary response gene 88 (MyD88), was first identified to be essential for the induction of inflammatory cytokines, including TNF- $\alpha$  and IL-12 [34]. However, subsequent research reports have indicated that individual TLR signaling pathways are divergent, and there are MyD88-dependent and TIR-domain-containing adaptor protein-inducing IFN- $\beta$  (TRIF)-dependent pathways. The variance of immune responses mediated by individual TLR ligands might be explained by the selective usage of these adaptor molecules. MyD88 and TRIF activate individual signaling pathways, causing the production of pro-inflammatory cytokines and type I IFNs, respectively [9].

## 1. MyD88-Dependent Pathway

MyD88 possesses a C-terminal TIR domain and an N-terminal death domain, and it relates to the TIR domain of TLRs. MyD88 intercedes with the activation of NF- $\kappa$ B and subsequent induction of inflammatory cytokine genes through all TLRs, except for TLR3. TRIF intercedes with TLR3- and TLR4-dependent activations of interferon regulatory factor (IRF) 3 and NF- $\kappa$ B, and subsequent induction of IFN- $\beta$  [35]. In plasmacytoid DCs (pDCs), MyD88 is also responsible for TLR7- and TLR9-dependent activations of IRF7, which is a vital factor for IFN- $\alpha/\beta$  induction [9,36].

## 2. TRIF-Dependent Pathway

This pathway, a MyD88-independent pathway that originates from TLR3 and TLR4, induces type I IFNs through the activation of IRF3 [37]. TRIF is vital for TLR3- and TLR4-mediated activations of IRF3, as it interacts with receptor-interacting protein 1, which causes activation of TRIF-dependent NF- $\kappa$ B [38,39].

## TLR-BASED DRUG DEVELOPMENT

Opportunities for TLR-based drug development are provided by TLR agonists and antagonists. Pharmacological agents (TLR agonists) contain synthetic lipopeptide derivatives from bacterial lipoproteins, ssRNA, CpG motif-containing oligonucleotides or CpG oligodeoxynucleotides. Patients who are susceptible to UTI, in which defective TLR mutants might be responsible for increased infection rates, could be treated with specific TLR-modulating drugs to conquer impaired immune defense systems, particularly in chronic recurrent UTI [40].

## 1. TLR Agonists

Therapeutic protocols for chronic recurrent UTI may blend TLR immunotherapy with chemotherapeutic agents, which make attempts to enhance the clearing of tissue-invasive uropathogenic bacteria. Stimulation of TLR-related antimicrobial signaling of target cells (monocytes, DCs, lymphocytes) via immunoadjuvants may reflect an innovative form of immunization, similar to—or even more effective than—the conventional vaccination that targets the pathogen [41]. In a similar method, recent pre-clinical studies used TLR ligands to treat viral diseases and to improve antitumor treatment (e.g., ligands that play as agonists for

TLR7 activation against cancer cells) [40].

## 2. TLR Antagonists

Recent therapeutic options for chronic recurrent UTI may contain strategies to modulate TLR activation and signaling to support conventional antibiotic treatment, particularly in clinical setting to increase antimicrobial resistance. Conversely, hyperinflammation and urosepsis can be suppressed by TLR antagonists [42], as observed in pro-inflammatory subsets of monocytes that are specifically sensitive to glucocorticoids [40].

## CONCLUSIONS

TLRs act an instructive role in UTI. TLRs, expressed both by epithelial and non-epithelial cells, launch appropriate immune and inflammatory responses to get over microbial invasion and infection. Synthetic TLR agonists and TLR antagonists affect and manipulate the host defense systems. Some of these immunomodulating drugs may help to conquer intrinsic disturbances in the TLR mechanism, providing new therapeutic options for UTI.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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