Review

Discovery Medicine 2026; 38(211): 2338–2352
https://doi.org/10.24976/Discov.Med.202638211.184

Toll-like Receptor Signaling Beyond Infection in Urinary Bladder Dysfunction: Molecular Insights and Therapeutic Implications

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Submitted: 1 June 2026 Revised: 27 June 2026 Accepted: 13 July 2026 Published: 24 August 2026

Lower urinary tract dysfunction is highly prevalent and imposes a substantial individual and societal burden. Yet, current pharmacotherapies offer only limited symptomatic relief and are constrained by adverse effects and poor adherence. Toll-like receptors (TLRs), and Toll-like receptor 4 (TLR4) in particular, are key pattern-recognition receptors of the innate immune system. Their role in bladder host defense against uropathogens is well established; more recently, however, the same receptors have been recognized as drivers of sterile, infection-independent inflammation by sensing endogenous damage-associated molecular patterns. This review focuses on that infection-independent role, synthesizing recent experimental evidence that implicates TLR signaling, acting largely through the NF-κB axis and its coupling to the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, in three major bladder disorders: interstitial cystitis/bladder pain syndrome (IC/BPS), bladder outlet obstruction (BOO), and diabetic bladder dysfunction (DBD). In IC/BPS, TLR4 mediates bladder pain independently of inflammation, while TLR7 has been implicated in the Hunner-type subtype; in BOO, TLR4 and TLR9 contribute to the early inflammatory phase of remodeling; and in DBD, TLR4 activation drives bladder hypertrophy and hypercontractility. Selective antagonists such as TAK-242 and inhibition of TLR4/NF-κB signaling attenuate these changes, identifying the pathway as a tractable therapeutic target. We concentrate on these three conditions because direct experimental evidence for TLR involvement is, to date, largely confined to them. Throughout, we distinguish human, animal, and *ex vivo* or cell-based findings, and we note that the supporting evidence remains most robust for IC/BPS and largely preliminary for BOO. We close by highlighting the field's near-exclusive focus on TLR4 and the mechanistic and translational gaps that future studies must address.

Keywords: bladder dysfunction; BOO; DBD; interstitial cystitis/bladder pain syndrome; sterile inflammation; TLR4

Introduction

Lower urinary tract dysfunction ranks among the most prevalent chronic health burdens worldwide. Population-based surveys applying standardized International Continence Society (ICS) definitions report that most adults experience at least one lower urinary tract symptom (LUTS) [1,2], and the pooled global prevalence of overactive bladder (OAB) is approximately 20% and rising [3]. At the global level, the broader burden of LUTS, urinary incontinence, and bladder outlet obstruction affects a substantial and rising share of the adult population, with the steepest increases concentrated in developing regions [4]. Functionally, bladder dysfunction spans overactive phenotypes, marked by urgency and frequency, and underactive phenotypes characterized by impaired bladder emptying [5,6]. This functional spectrum spans several distinct yet mechanistically overlapping disorders of substantial individual and societal cost, including interstitial cystitis/bladder pain syndrome (IC/BPS), reported in approximately 2.7–6.5% of adult women [7] and 1.9–4.2% of men [8] in the United States; bladder outlet obstruction (BOO); and diabetic bladder dysfunction, with an estimated prevalence of 25–87% [9]. Despite this high prevalence, currently available pharmacotherapies provide only limited and often incomplete symptom relief, and their use is frequently constrained by mechanism-based adverse effects, including dry mouth, constipation, and cognitive impairment, as well as by poor long-term adherence and persistence [10]. These limitations underscore a pressing need for novel therapeutic strategies that target the underlying pathophysiology of bladder dysfunction rather than its symptoms alone.

Toll-like receptors (TLRs), and TLR4 in particular, are key pattern-recognition receptors (PRRs) of the host innate immune system [11]. Although best known for recognizing lipopolysaccharide (LPS) from Gram-negative bacteria to initiate the inflammatory response, TLR4 has more recently been established as a central effector in the pathogenesis of sterile inflammation, chronic pain, and metabolic disease [12]. In keeping with this broader role,

TLR4 in bladder pathology has become a rapidly expanding area of investigation over the past decade, with accumulating evidence implicating the receptor in three major bladder dysfunctions: IC/BPS, BOO, and diabetic bladder dysfunction (DBD). A causal contribution has been established in IC/BPS, in which genetic deletion or selective antagonism of TLR4 attenuates bladder pain independently of inflammation and voiding dysfunction [13], and in DBD, in which TLR4 activation drives bladder hypertrophy and hypercontractility [14]. In BOO, TLR4 and TLR9 are concurrently upregulated and co-localize with α1-adrenergic receptors, implicating them in the early inflammatory phase of obstruction-induced remodeling [15]. Mechanistically, TLR4 activation promotes pelvic nociception through spinal glial activation and cytokine signaling, independently of changes in voiding patterns [13]. From a therapeutic perspective, selective TLR4 antagonists such as TAK-242 (resatorvid) attenuate bladder nociception and sensory hypersensitivity [13,16], whereas inhibition of TLR4/NF-κB signaling limits bladder inflammation [17]. By synthesizing the most recent and influential studies in this area, the present review aims to elucidate the molecular mechanisms underlying TLR-mediated bladder dysfunction and evaluate TLRs as therapeutic targets for management.

TLR Signaling Pathway in Bladder

Canonical TLR Signaling

TLRs are germline-encoded pattern-recognition receptors (PRRs) and constitute the most thoroughly characterized sensors of the innate immune system [18,19]. Structurally, each receptor comprises an ectodomain of horseshoe-shaped leucine-rich repeats (LRRs) that mediates ligand recognition and a cytoplasmic Toll/interleukin-1 receptor (TIR) domain that nucleates downstream signaling [18]. Among the mammalian TLRs, TLR4 is the principal sensor of bacterial lipopolysaccharide (LPS) and the receptor most relevant to the urinary tract [20].

TLRs recognize conserved pathogen-associated molecular patterns (PAMPs) that are shared among microbes but absent from the host, as well as endogenous damage-associated molecular patterns (DAMPs) released from injured or dying cells [19]. Among the surface receptors, TLR4 recognizes LPS from Gram-negative bacteria; TLR2, in association with TLR1 or TLR6, senses a broad range of lipoproteins, peptidoglycans, and lipoteichoic acids; and TLR5 detects bacterial flagellin [20]. The endosomal receptors recognize nucleic acids, with TLR3 sensing viral double-stranded RNA, TLR7 and TLR8 sensing single-stranded RNA, and TLR9 recognizing unmethylated CpG DNA motifs of bacterial and viral origin [18]. In addition to microbial ligands, endogenous danger signals such as heat-shock proteins and hyaluronan fragments can engage TLR4 and TLR2, thereby driving sterile inflammation. The failure of TLRs to discriminate self from non-self, or their aberrant activation by self-derived ligands, contributes to autoimmune and chronic inflammatory disease [19].

Signal transduction begins when ligand binding promotes TLR dimerization and the recruitment of TIR domain-containing adaptor proteins to the cytoplasmic TIR domain of the receptor. Four principal adaptors have been defined, namely MyD88, TIRAP (also called Mal), TRIF, and TRAM, and their differential usage provides specificity to individual receptor cascades. MyD88 serves as the universal adaptor employed by all TLRs except TLR3. TIRAP functions as a sorting adaptor that recruits MyD88 to cell-surface receptors such as TLR2 and TLR4, whereas TRAM is selectively required to bridge TRIF to TLR4 [20]. Based on adaptor recruitment, TLR signaling is broadly divided into the MyD88-dependent pathway, common to nearly all TLRs, and the MyD88-independent or TRIF-dependent pathway, which is restricted to TLR3 and TLR4 [19].

In the MyD88-dependent pathway, MyD88 is recruited to the activated receptor and assembles with members of the IRAK kinase family to form a complex known as the Myddosome. Within this complex, IRAK4 phosphorylates and activates IRAK1, which then undergoes autophosphorylation and dissociates from MyD88 [18]. Activated IRAK1 associates with the RING-domain E3 ubiquitin ligase TRAF6, which, together with the ubiquitin-conjugating enzymes Ubc13 and Uev1A, catalyzes K63-linked polyubiquitination of TRAF6 itself and of the TAK1 kinase complex [20]. TAK1, a member of the MAPKKK family, forms a complex with the regulatory subunits TAB1, TAB2, and TAB3, and its activation then diverges into two arms [18]. In the first arm, TAK1 activates the IKK complex, composed of the catalytic subunits IKKα and IKKβ and the regulatory subunit NEMO (IKKγ), which phosphorylates the inhibitory protein IκB and targets it for proteasomal degradation, thereby releasing NF-κB to translocate into the nucleus and induce proinflammatory genes. In the second arm, TAK1 activates the MAP kinase cascade, leading to activation of the transcription factor AP-1 [18]. Acting together, NF-κB and AP-1 drive the production of proinflammatory cytokines such as TNF-α, IL-6, and IL-12 [19].

TLR3 and TLR4 engage the MyD88-independent pathway and proceed through the adaptor TRIF [20]. Whereas TLR3 binds TRIF directly, TLR4 requires the bridging adaptor TRAM to associate TRIF with the receptor. TRIF recruits TRAF3, which in turn engages the non-canonical kinases TBK1 and IKKε to phosphorylate the transcription factor IRF3. Phosphorylated IRF3 dimerizes and translocates to the nucleus, where it drives expression of the type I interferon IFN-β, and IFN-β subsequently activates STAT1 to induce a range of interferon-inducible genes important for antiviral defense [18]. This pathway also contributes to a delayed, late-phase activation of NF-κB [19]. Because TLR4 signals through both adaptor sys-

tems, full induction of its inflammatory cytokine response requires activation of both the MyD88-dependent and the TRIF-dependent pathways [20].

Because the unchecked production of proinflammatory cytokines can precipitate severe systemic disorders, TLR signaling is tightly constrained by a large set of negative regulators that act at each tier of the cascade [19]. These include molecules that prevent the adaptors MyD88 and TRIF from binding their partners, deubiquitinating enzymes and other inhibitors that target TRAF6 and TAK1, and transcriptional regulators that suppress NF-κB and IRF3, which together limit excessive innate immune activation and protect the host from autoimmune and inflammatory injury.

Bladder Tissue-specific TLR Expression and the cAMP/CREB Pathway

Within the urinary tract, TLR4 is the most extensively characterized receptor and, together with its co-receptor CD14, is amply expressed on the superficial umbrella cells lining the bladder lumen in both mice and humans, where it provides continuous immunosurveillance of the bladder milieu [21]. The receptors encountered in the urinary tract also include TLR2, TLR3, TLR5, TLR9, and TLR11, but TLR4 is the best-studied and the principal driver of the cytokine and chemokine response to Gram-negative bacteria [22]. Upon recognition of bacterial LPS, TLR4/CD14 complexes on bladder epithelial cells (BECs) initiate the classical signaling pathway, recruiting the TIR adaptors TIRAP/Mal and MyD88, along with the downstream substrates IRAK and TRAF6, to activate the transcription factor NF-κB. NF-κB then translocates to the nucleus and stimulates the transcription of cytokines, including IL-6 and IL-8, which are among the principal mediators released in the urinary tract following bacterial infection. In addition to the classical NF-κB axis, BECs possess a second, parallel TLR4-activated pathway that also contributes to the IL-6 and IL-8 responses [21]. In this pathway, ligation of TLR4 triggers an influx of intracellular calcium followed by a rapid rise in cyclic AMP (cAMP) generated by adenylyl cyclase 3, the specific isoform responsible for cAMP production in human BECs. The increase in cAMP, acting through protein kinase A, leads to phosphorylation of the cAMP response element-binding protein (CREB), which upregulates the expression of IL-6 and IL-8 [21]. Although LPS ligation activates both pathways, the kinetics of IL-6 secretion through the cAMP/CREB pathway are markedly faster, by roughly two hours, than through the classical pathway, an adaptation that may allow the bladder to respond promptly to the constant influx of contaminants from the adjacent gut flora [22]. Beyond cytokine production, the TLR4/cAMP axis confers additional bladder-specific antimicrobial activities on BECs [21]. Through the downstream effector protein kinase A, intracellular cAMP inhibits Rac-1, a lipid-raft-associated component required for the actin remodeling that underlies bacterial entry. It thereby suppresses the invasion of BECs by uropathogenic bacteria. The magnitude of this defense is illustrated by the finding that invasion of the bladder by type 1 fimbriated Escherichia coli or Klebsiella pneumoniae is 10- to 12-fold greater in TLR4-mutant (C3H/HeJ) mice than in wild-type animals. The same TLR4-initiated, cAMP-dependent signaling further enables BECs to expel bacteria that have already invaded. Internalized bacteria are housed within fusiform vesicles, a population of cAMP-regulated exocytic vesicles that normally regulate bladder volume, and the rise in cAMP triggered by TLR4 promotes the exocytosis of these vesicles and the piecemeal expulsion of the bacteria they contain. Consistent with this mechanism, pharmacologically increasing intracellular cAMP with the agent forskolin reduces the bacterial burden in infected murine bladders, suggesting that activators of the TLR4 signaling pathway may hold therapeutic value against urinary tract infection [22]. Although TLR4 predominates, other receptors contribute to bladder immunosurveillance. TLR5, the sensor of bacterial flagellin, is expressed at high levels on bladder epithelial cells and drives a vigorous chemokine response, whereas TLR11 is localized mainly to the kidney rather than the bladder and is not functionally expressed in humans owing to a premature stop codon in its open reading frame [21]. Collectively, these canonical and bladder-specific TLR4 signaling pathways are summarized in Fig. 1.

TLR Signaling in Bladder Dysfunction

Although TLRs were originally defined by their role in host defense against invading pathogens, it is now recognized that the same receptors, particularly TLR4, drive sterile inflammation through recognition of endogenous DAMPs released from stressed or injured tissue, independently of microbial infection [11,12]. TLR4 also serves as the principal sensor of uropathogens in the bladder, where it orchestrates the antibacterial cytokine response [22]; however, the present review deliberately focuses on its more recently recognized, infection-independent role in sterile inflammation and bladder dysfunction. In the lower urinary tract, this capacity has positioned TLR4 as a candidate link between local tissue injury and the chronic inflammatory and functional changes that characterize several bladder disorders [12]. Over the past decade, accumulating experimental evidence has implicated TLR signaling, acting largely through the NF-κB axis, in the pathogenesis of three major forms of bladder dysfunction: IC/BPS, BOO, and DBD [13,14,15] (Table 1, Ref. [13,14,15,16,17,23,24,25,26,27,28,29,30,31,32,33,34,35], Fig. 2). In these settings, TLR4 activation has been associated with distinct disease-relevant outcomes, ranging from bladder pain that arises independently of overt inflammation [13] to the early inflammatory remodeling of the obstructed bladder [15] and the hypertrophy and

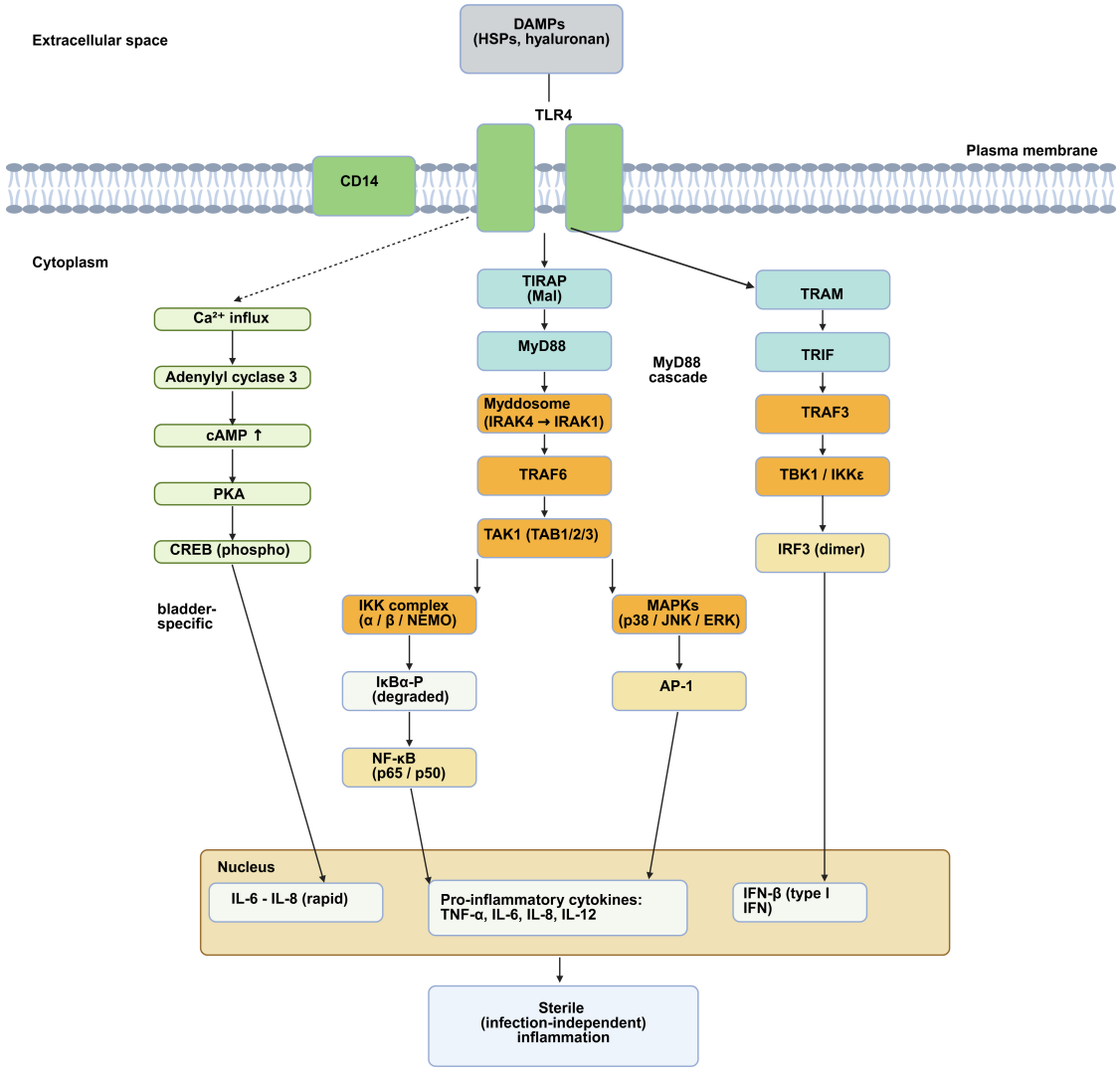

Fig. 1. TLR4 signaling in bladder epithelial cells and its infection-independent inflammatory output. This review focuses on the sterile, infection-independent activity of TLR4. Beyond its classical role in antibacterial defense, the receptor is engaged by endogenous damage-associated molecular patterns (DAMPs), such as heat-shock proteins and hyaluronan fragments released during tissue stress or injury. DAMP-engaged TLR4 (with its co-receptor CD14) signals through two adaptor arms. The MyD88-dependent arm (TIRAP/Mal and MyD88 → Myddosome [IRAK4/IRAK1] → TRAF6 → TAK1 [TAB1/2/3]) activates the IKK complex (IκBα degradation → NF-κB) and the MAPK cascade (→ AP-1), which together drive transcription of proinflammatory cytokines (TNF-α, IL-6, IL-8, IL-12). The TRIF-dependent arm (TRAM and TRIF → TRAF3 → TBK1/IKKε → IRF3) induces the type I interferon IFN-β. Bladder epithelial cells additionally possess a tissue-specific TLR4 pathway, originally described in the context of antimicrobial defense, that couples the receptor to Ca^{2+} influx, adenylyl cyclase 3, cAMP, PKA and CREB and provides a rapid, parallel route to IL-6 and IL-8 production. Converging on NF-κB-driven cytokine output, these pathways underlie the sterile, infection-independent inflammation implicated in bladder dysfunction. Solid arrows indicate direct activation or sequential signaling steps; the dashed arrow indicates an indirect link; the thin line without an arrowhead denotes ligand binding. (Created with BioRender.com, License Number: SZ29YMIET3). TLR4, Toll-like receptor 4; TIRAP, TIR domain-containing adaptor protein; IKK, IκB kinase (inhibitor of nuclear factor κB kinase); AP-1, activator protein 1; TRAM, TRIF-related adaptor molecule; TRIF, TIR domain-containing adaptor inducing interferon-β; cAMP, cyclic AMP; PKA, protein kinase A; CREB, cAMP response element-binding protein.

hypercontractility that accompany the diabetic bladder [14]. The following sections examine the evidence for TLR signaling in each of these conditions in turn and consider the molecular mechanisms through which the pathway contributes to bladder dysfunction.

Table 1. TLR signaling in bladder dysfunction: summary of key evidence.

Disease type	Main receptor	Experimental system	Stimuli / inducers	Intervention (drugs / methods)	Core results	Evidence characteristics (species; sample type; evidence level)	Ref.
IC/BPS	TLR4	Human cohort (*ex vivo* PBMC)	LPS (TLR4 agonist)	None (observational)	*Ex vivo* LPS-evoked IL-1β and IL-6 release correlates with pain severity	Human; PBMC; clinical, observational	[28]
	TLR4	Human cohort (*ex vivo* PBMC)	LPS (TLR4 agonist)	None (observational)	Stronger TLR4-evoked response linked to widespread pain and comorbid syndromes	Human (n = 66); PBMC; clinical, observational	[29]
	TLR4	Human cohort (whole blood)	LPS (TLR4 agonist)	None (observational)	Greater life adversity associated with a higher TLR4 inflammatory composite	Human (n = 154 + 32 controls); whole blood; clinical, observational	[30]
	TLR4	Mouse, *in vivo* (intravesical)	Disulfide HMGB1 (DAMP)	TAK-242; RAGE antagonist	TAK-242 abolished HMGB1-evoked bladder pain without inflammation; RAGE antagonist ineffective locally	Mouse; bladder; preclinical, causal (pharmacological)	[31]
	TLR4	Mouse, *in vivo*	CYP (acrolein)	TAK-242; TLR4 knockout	Prevented voiding dysfunction and inflammation; normalized contractility and M2/M3 receptor expression	Mouse; bladder; preclinical, causal (genetic + pharmacological)	[16]
	TLR4	Mouse, *in vivo*	CYP	Trimetazidine	Reduced bladder TLR4 and p-NF-κB, lowered TNF-α / IL-1β, improved detrusor contractility	Mouse; bladder; preclinical, interventional (pharmacological)	[32]
	TLR4	Mouse, *in vivo* (URO-OVA transgenic)	Autoimmune (URO-OVA)	TAK-242; TLR4 knockout	Reduced nociception independently of bladder inflammation and voiding dysfunction	Mouse (female); bladder, spinal cord; preclinical, causal (genetic + pharmacological)	[13]
	TLR4	Mouse, *in vivo* + astrocyte culture	Protamine / LPS	MSC-derived exosomes (miR-9)	Reduced nociception and microgliosis via suppression of spinal TLR4 / NLRP3 signaling	Mouse; spinal cord, bladder; preclinical, interventional (mechanistic)	[33]
	TLR4	Rat, *in vivo*	CYP	Baicalin	Suppressed TLR4 / MyD88 / NF-κB p65; reduced serum TNF-α / IL-6 / IL-1β and tissue damage	Rat; bladder, serum; preclinical, interventional (pharmacological)	[27]
	TLR4	Rat, *in vivo*	CYP	Platelet-rich plasma	Improved bladder injury through TLR4 / NF-κB; restored urothelial barrier (ZO-1)	Rat; bladder; preclinical, interventional	[17]
	TLR4	Mouse, *in vivo*	CYP	Pramipexole + lactoferrin	Inhibited TLR4 / NF-κB and NLRP3 / caspase-1 / IL-1β; enhanced Nrf2 / HO-1	Mouse; bladder; preclinical, interventional	[23]
	TLR4	Mouse, *in vivo*	CYP	Fosaprepitant (NK1R antagonist)	Partly reduced bladder inflammation, TLR4 expression and MPO activity; improved detrusor overactivity	Mouse; bladder; preclinical, interventional	[26]
	TLR4	Mouse, *in vivo* + cell culture	LPS	THX-B (p75NTR antagonist)	Reduced urothelial TNF-α and TLR4 / TRAF6 / NF-κB-driven bladder inflammation	Mouse; bladder, urothelial / smooth-muscle cells; preclinical, interventional (*in vitro* + *in vivo*)	[25]
	TLR7	Human biopsy + mouse, *in vivo*	ssRNA / loxoribine (TLR7 agonist)	Hydroxychloroquine (TLR7 antagonist)	TLR7 overexpressed in Hunner-type IC; agonist induced cystitis and pain, antagonist reversed it	Human (biopsy) + mouse; bladder; translational (clinical expression + preclinical causal)	[34]

Table 1. Continued.

Disease type	Main receptor	Experimental system	Stimuli / inducers	Intervention (drugs / methods)	Core results	Evidence characteristics (species; sample type; evidence level)	Ref.
BOO	TLR4 and TLR9	Rat, *in vivo* (partial BOO)	Outlet obstruction (ischemia / DAMPs)	Tamsulosin (α1 antagonist)	TLR4 and TLR9 upregulated and co-localized with α1-adrenergic receptors in the inflammatory phase; tamsulosin lowered urinary IL-6 / IL-18 without changing TLR expression	Rat; bladder, urine; preclinical, associative (expression + co-localization; no causal test)	[15]
DBD	TLR4	Mouse, *in vivo* + *ex vivo* strips	STZ diabetes / HMGB1 (DAMP)	CLI-095; TLR4 knockout	Prevented bladder hypertrophy and hypercontractility despite comparable hyperglycemia; normalized *ex vivo* contractility	Mouse; bladder; preclinical, causal (genetic + pharmacological + *ex vivo*)	[14]
	NLRP3 (TLR4-coupled)	Narrative review	Diabetic DAMPs (meta-inflammation)	None (review)	NLRP3 inflammasome (coupled to TLR4) proposed as a central driver of DBD and its progression toward an underactive bladder	Not applicable; narrative review; expert synthesis	[24]
Radiation cystitis	TLR4	Rat, *in vivo*	Bladder irradiation (20 Gy)	None (observational)	Downregulated urothelial TLR4 and IL-6 and reduced submucosal mast cells, with altered urodynamics; contrasting down-regulation vs the three principal forms	Rat; bladder; preclinical, observational (expression)	[35]

Abbreviations: BOO, bladder outlet obstruction; CYP, cyclophosphamide; DAMP, damage-associated molecular pattern; DBD, diabetic bladder dysfunction; HMGB1, high-mobility group box 1; HO-1, heme oxygenase-1; IC, interstitial cystitis; IC/BPS, interstitial cystitis/bladder pain syndrome; IL, interleukin; LPS, lipopolysaccharide; M2/M3, muscarinic acetylcholine receptor subtypes 2 and 3; miR, microRNA; MPO, myeloperoxidase; MSC, mesenchymal stem cell; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor-κB; NK1R, neurokinin 1 receptor; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; Nrf2, nuclear factor erythroid 2-related factor 2; p-, phosphorylated; p65, NF-κB p65 subunit; p75NTR, p75 neurotrophin receptor; PBMC, peripheral blood mononuclear cell; RAGE, receptor for advanced glycation end products; ssRNA, single-stranded RNA; STZ, streptozotocin; TLR, Toll-like receptor; TNF-α, tumor necrosis factor-α; TRAF6, TNF receptor-associated factor 6; URO-OVA, urothelial ovalbumin-expressing transgenic mouse (autoimmune cystitis model); ZO-1, zonula occludens-1.

TLR Cross-Talk and the Bladder Inflammatory Network

Across the disorders discussed below, TLR signaling does not operate in isolation but functions as the hub of an interconnected inflammatory network, and several recurring cross-talk nodes are best considered together. The first and most consistent is the TLR4/NF-κB axis itself, which is engaged in IC/BPS, BOO, and DBD alike and provides the common route from receptor activation to the transcription of TNF-α, IL-6, and IL-1β [13,14,16]. A second node couples TLR4 to the NLRP3 inflammasome. NF-κB-dependent priming licenses NLRP3, which, once activated, matures IL-1β and can drive urothelial pyroptosis, a circuit invoked in both cyclophosphamide (CYP) cystitis and diabetic bladder dysfunction [23,24,36,37]. A third layer involves the pro-inflammatory neurotrophin receptor p75NTR, which shares the adaptor TRAF6 with TLR4 and converges on NF-κB and JNK; its blockade attenuates TLR4-driven urothelial inflammation [25]. Neurogenic signaling provides a fourth node, as substance P, acting through the neurokinin-1 receptor (NK1R), upregulates bladder TLR4, linking sensory neurotransmission to innate immune activation, while NK1R antagonism reduces both TLR4 expression and detrusor overactivity [26]. Finally, these pro-inflammatory circuits are counter-balanced by the cytoprotective Nrf2/HO-1 axis, whose pharmacological induction suppresses TLR4/NF-κB and NLRP3 signaling and limits oxidative injury [23]. Viewed together, these interactions place TLR4 at the intersection of innate immune, inflammasome, neurotrophic, neurogenic, and antioxidant pathways, and the disease-specific findings summarized in the following sections represent different facets of this shared network.

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS)

IC/BPS is a chronic, non-infectious inflammatory disorder of the bladder wall characterized by suprapubic or pelvic pain accompanied by lower urinary tract symptoms such as urinary frequency, urgency, and occasionally incontinence [13,16]. Because no disease-specific biomarker is currently available, diagnosis relies largely on the exclusion of other conditions presenting with a similar clinical picture [38,39]. The disorder is considerably more common in women than in men, with reported prevalences in the United States of approximately 2.7–6.5% in women and 1.9–4.2% in men [7,8]. Its etiopathogenesis is multifactorial and remains incompletely understood, with urothelial barrier dysfunction and consequent increased bladder permeability, aberrant immune activation, neurogenic inflammation, mast cell activation, and altered neural signaling among the principal mechanisms proposed [40]. A common feature of these processes is a chronic inflammatory state sustained by overexpression of pro-inflammatory cytokines, most notably TNF-α, IL-6, and IL-1β; in the absence of a curative treatment, the disease typically follows a recurrent course that severely impairs quality of life [27,41].

TLRs are highly conserved pattern-recognition receptors of the innate immune system that detect microbe-associated molecular patterns and damage-associated molecular patterns released from injured tissue [11]. TLR4 recognizes LPS from Gram-negative bacteria, which are the most frequent cause of bacterial cystitis. It signals through the adaptor proteins MyD88 and TRIF, driving phosphorylation and nuclear translocation of NF-κB, culminating in the release of pro-inflammatory cytokines such as TNF-α and IL-1β [12,16]. Because TLR4 is expressed on bladder epithelial cells and on phagocytic cells recruited during infection, TLR signaling has been implicated in a range of urinary tract pathologies, including urinary tract infection, bladder cancer, bladder outlet obstruction, and bladder dysfunction [14,15,22]. Among pattern-recognition receptors, TLR4 has been the most extensively implicated in IC/BPS.

Clinical Evidence

In patients with IC/BPS, peripheral blood mononuclear cells (PBMCs) produce elevated levels of pro-inflammatory cytokines such as IL-1β and IL-6 when stimulated *ex vivo* with the TLR4 agonist LPS, and the magnitude of this response correlates with pain severity, pointing to a role for TLR4-mediated inflammatory signaling in the disorder [28]. In a study of 66 women with IC/BPS whose PBMCs were stimulated with TLR2 and TLR4 agonists, a stronger TLR4-evoked inflammatory response was associated with a greater likelihood of pain extending beyond the pelvis, as well as with greater self-reported pain severity and interference. This response was higher in patients with a comorbid syndrome than in those with IC/BPS alone, distinguished patients with comorbid irritable bowel syndrome, temporomandibular disorder, and vulvodynia from those with IC/BPS only, and increased with the number of comorbid conditions [29]. Notably, while TLR2 reactivity has been shown to distinguish IC/BPS patients from healthy controls, it did not differ between patients with and without comorbid syndromes, suggesting that TLR2 and TLR4 sensitivities reflect distinct facets of the disease [28,29]. A subsequent, larger Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) study examined the determinants of TLR4-stimulated inflammation in 154 women with IC/BPS and 32 healthy controls, using whole-blood LPS stimulation to quantify seven cytokines and chemokines (IL-6, TNF-α, IL-1β, MIP-1α, MCP-1, IL-8, and IL-10) that were reduced to a global TLR4 inflammatory composite by principal components analysis. Greater exposure to recent life adversity of at least moderate severity, as well as cumulative early and recent adversity, was associated with a higher composite score.

In contrast, early life adversity alone showed only a non-significant trend [30]. As in the earlier cohort, overall

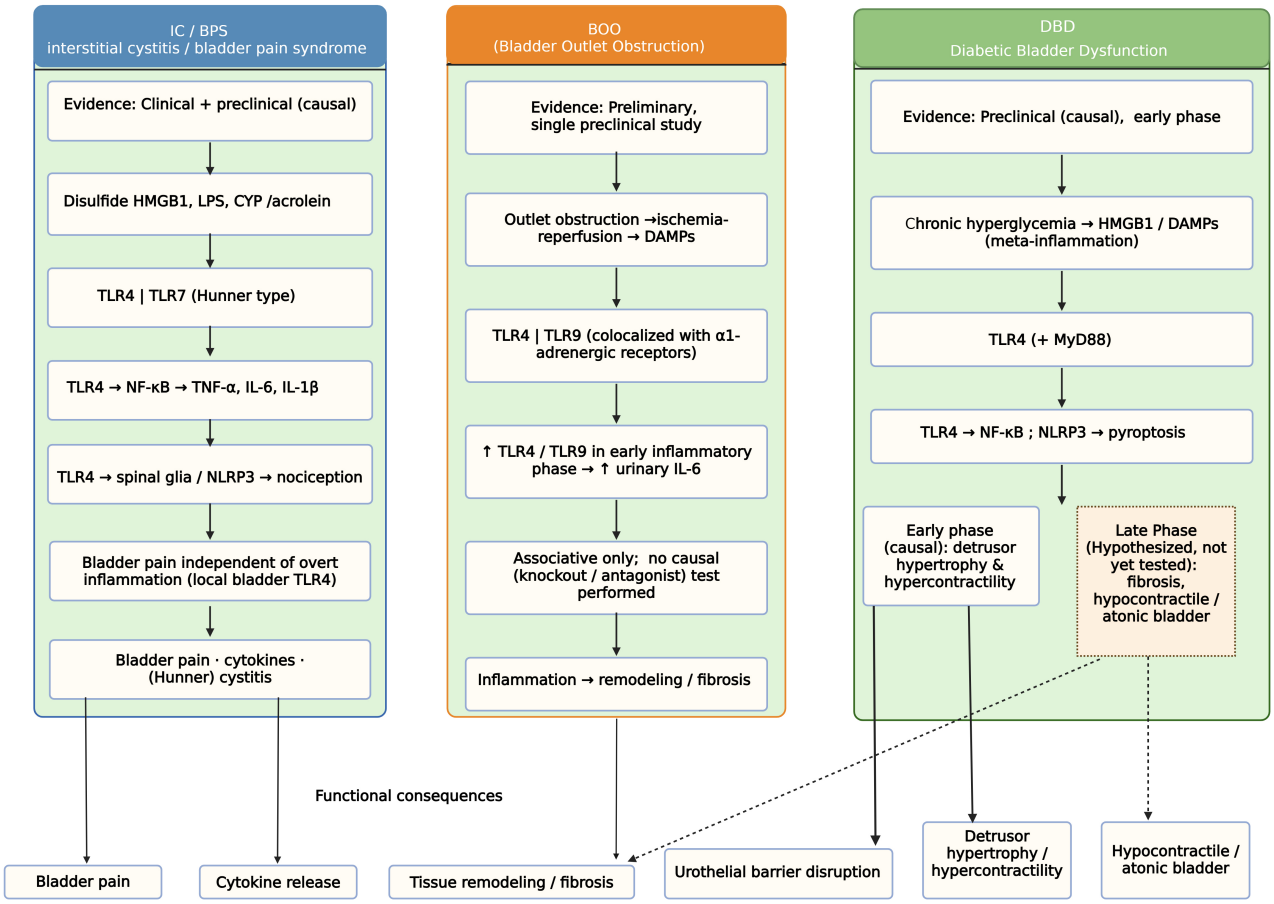

Fig. 2. TLR signaling as a convergent mechanism linking sterile inflammation to bladder dysfunction across IC/BPS, BOO, and DBD, with the strength of evidence indicated for each condition. In IC/BPS (clinical and causal preclinical evidence), sterile ligands (disulfide HMGB1, LPS, cyclophosphamide/acrolein) engage TLR4 and TLR7 in the Hunner-type subtype, driving NF-κB-dependent cytokine production (TNF-α, IL-6, IL-1β) and spinal glial/NLRP3-mediated nociception, including bladder pain that can arise independently of overt inflammation through local bladder TLR4. In BOO (preliminary, single preclinical study), outlet obstruction causes ischemia-reperfusion and DAMP release, and TLR4 and TLR9 (colocalized with α1-adrenergic receptors) are upregulated during the early inflammatory phase, with increased urinary IL-6; this evidence is associative only, with no causal test (knockout or antagonist) performed. In DBD (causal preclinical evidence, early phase), chronic hyperglycemia generates HMGB1/DAMPs (meta-inflammation) that activate TLR4/MyD88/NF-κB and the NLRP3 inflammasome; the causally demonstrated early phase (solid arrows) produces detrusor hypertrophy, hypercontractility, and pyroptotic urothelial barrier disruption, whereas the late fibrotic, hypocontractile/atonic phase (dashed arrows) is hypothesized but not yet directly tested. Solid arrows denote demonstrated (causal or directly observed) links; dashed arrows denote hypothesized or not-yet-tested links. (Created with BioRender.com, License Number: PD29YMI1UD).

TLR4 immunoreactivity did not differ between patients and controls. Rather, TLR4 reactivity appears to be elevated specifically in patients with widespread pain or chronic overlapping pain conditions, rather than in those with localized pain, supporting the view that it tracks the emergence of nociplastic pain in IC/BPS [29,30]. Taken together, these clinical studies position TLR4-stimulated inflammation as one of the first replicable biological markers of pain phenotype in IC/BPS.

Mechanistic Insights From Preclinical Models

Beyond microbial LPS, an important endogenous TLR4 ligand in this context is the damage-associated molecular pattern protein high-mobility group box 1 (HMGB1). Its extracellular activity is redox-dependent, since the all-thiol form preferentially engages the receptor for advanced glycation end products (RAGE), whereas the disulfide form binds TLR4, driving cytokine production. In a murine model of bladder pain, intravesical instillation of disulfide HMGB1, but not the all-thiol form, produced abdominal mechanical hypersensitivity at a dose that did

not cause overt inflammation. This hypersensitivity was abolished by intravesical pretreatment with the TLR4 antagonist TAK-242 but not by a RAGE antagonist, whereas systemic administration of either antagonist was effective [31]. These findings indicate that disulfide-HMGB1 can directly and independently of inflammation evoke bladder pain through TLR4 in the bladder itself, identifying local bladder TLR4 as a specific target for pain relief.

Preclinical models of cystitis have provided some of the most direct mechanistic evidence for TLR4. In the CYP-induced model, in which the urotoxic metabolite acrolein drives oxidative stress and inflammation, bladder mRNA expression of TLR4, MyD88, and TRIF is markedly increased. Either genetic deletion of TLR4 or its pharmacological blockade with TAK-242 prevents the associated urodynamic disturbances, including increased micturition frequency, non-voiding contractions, reduced bladder capacity, and shortened intercontraction interval. TLR4 suppression also normalizes impaired bladder contractility to carbachol, reduces mRNA levels of M2 and M3 muscarinic receptors, and lowers myeloperoxidase activity, along with IL-6 and TNF-α levels, establishing TLR4 as a central initiator of both the voiding dysfunction and the inflammation that characterize CYP-induced cystitis [16]. Consistent with these observations, the anti-anginal drug trimetazidine, evaluated as a uroprotective agent in the same model, reduced the CYP-induced increase in bladder TLR4 and phosphorylated NF-κB protein expression without altering TLR2 expression. It concurrently lowered TNF-α, IL-1β, and the pro-apoptotic marker caspase-3, restored antioxidant capacity, and improved detrusor contractility, providing the first evidence that TMZ attenuates CYP-induced cystitis, at least in part, through downregulation of TLR4-mediated NF-κB signaling [32]. By contrast, the role of TLR4 appears more selective in the transgenic autoimmune cystitis (URO-OVA) model. Following cystitis induction, URO-OVA mice developed pelvic and bladder pain, along with increased splenocyte production of TLR4-mediated IL-1β, IL-6, and TNF-α and elevated spinal expression of IL-6, TNF-α, the glial activation markers CD11b and GFAP, and the endogenous TLR4 ligand HMGB1. Notably, TLR4-deficient URO-OVA mice showed markedly reduced nociceptive responses despite developing comparable bladder inflammation and voiding dysfunction, and systemic administration of TAK-242 similarly attenuated nociception, indicating that in this model, TLR4 is critical for bladder nociception independently of inflammation and voiding dysfunction [13]. This divergence, whereby TLR4 governs both voiding dysfunction and inflammation in the chemically induced model yet only pain in the autoimmune one, most likely reflects differences between the two models in both the type of cystitis and the sex of the animals studied (male versus female). The contribution of TLR4 also extends to the central nervous system, where it has been linked to activation of the NLRP3 inflammasome. In a protamine/LPS-induced IC/BPS model, TLR4-mediated neuroinflammation was critical for bladder nociception, and TAK-242 alleviated pelvic and bladder nociception while reducing spinal HMGB1, GFAP, and IBA1. The microRNA miR-9, which targets the 3′UTR of TLR4 and suppresses its protein expression, was downregulated in the spinal cord of model mice and in LPS-treated astrocytes, implicating its loss in glial TLR4 activation. Accordingly, intrathecal delivery of mesenchymal stem cell-derived exosomes enriched in miR-9 reduced bladder nociception, frequent micturition, microgliosis, and both systemic and central TLR4-mediated inflammation, an effect attributed to suppression of the spinal NLRP3 inflammasome via inhibition of the TLR4/NF-κB pathway in the dorsal horn [33].

Therapeutic Interventions Targeting TLR Signaling

The TLR4/NF-κB axis has consequently emerged as a promising therapeutic target. In a CYP-induced rat model of IC/BPS, baicalin, a flavonoid derived from *Scutellaria baicalensis*, reduced urination frequency and bladder tissue damage and decreased serum TNF-α, IL-6, and IL-1β; integrating network pharmacology, metabolomics, molecular docking, and Western blotting, the study showed that baicalin formed stable complexes with TLR4, MyD88, and NF-κB p65 and dose-dependently suppressed their expression, thereby acting largely through inhibition of the TLR4/NF-κB pathway [27]. Consistent with this, platelet-rich plasma has been reported to improve CYP-induced IC in rats through the TLR4/NF-κB signaling pathway [17]. The repurposed agents pramipexole and lactoferrin, administered against CYP-induced hemorrhagic cystitis, inhibited TLR4/NF-κB signaling, lowered IL-6 and TNF-α, suppressed the NLRP3/caspase-1/IL-1β cascade, and concurrently enhanced the cytoprotective Nrf2/HO-1 pathway, with the anti-inflammatory action of lactoferrin attributed in part to direct binding of TLR4 [23]. Likewise, the neurokinin 1 receptor (NK1R) antagonist fosaprepitant, evaluated in CYP-induced cystitis, attenuated detrusor overactivity and the inflammatory response while partially reducing bladder TLR4 expression and myeloperoxidase activity, consistent with reported crosstalk in which substance P drives TLR4 upregulation through NK1R [26]. Finally, TLR4 signaling engages in crosstalk with the pro-inflammatory neurotrophin receptor p75NTR, both receptors converging on NF-κB and JNK through recruitment of TRAF6; in LPS-triggered inflammation of bladder cells, the p75NTR antagonist THX-B abolished the rise in urothelial TNF-α, prevented JNK phosphorylation, NF-κB translocation, and the TRAF6-p75NTR association in smooth muscle cells, and reduced LPS-induced bladder inflammation *in vivo* [25]. Taken together, these findings reinforce TLR4 and its coupling to NF-κB and the NLRP3 inflammasome, as a convergent and pharmacologically tractable node across chemically induced, autoimmune, and neuroinflammatory models of IC/BPS.

Subtype-Specific Considerations and Model Limitations

Although TLR4 dominates the literature on IC/BPS, attention to the bladder tissue itself has implicated a second receptor, TLR7, in Hunner-type interstitial cystitis (HIC), a subtype characterized by pancystitis, clonal B-cell expansion, and epithelial denudation that frequently overlaps with autoimmune disorders such as systemic lupus erythematosus and Sjögren syndrome. TLR7 recognizes single-stranded RNA from viruses or bacteria and triggers the release of inflammatory cytokines and type I interferon, and because it is expressed in neurons and glial cells as well as in immune cells, it has been linked to both inflammation and pain. In bladder biopsies from patients with HIC, the number of TLR7-immunoreactive cells was significantly higher than in control tissue, and TLR7 mRNA was markedly upregulated in the background mucosa, providing the first direct evidence of TLR7 overexpression in the HIC bladder, with the weaker signal in Hunner lesions attributable to urothelial denudation. In mice, intravesical instillation of the selective TLR7 agonist loxoribine increased mucosal TLR7 mRNA and produced edema, congestion, and suburothelial inflammatory cell infiltration, confirming that TLR7 activation is sufficient to elicit a cystitis-like phenotype. Loxoribine also induced bladder pain-like licking behavior, raised voiding frequency, reduced voided volume, and facilitated mechanosensitive afferent nerve activity, indicating bladder sensory hyperactivity, whereas the TLR7 antagonist hydroxychloroquine reversed these voiding and cystometric changes [34]. These findings led the authors to propose TLR7 blockade as a potential therapeutic target for HIC.

Taken together, these studies establish TLR signaling, chiefly through TLR4 and its coupling to NF-κB, as a convergent mechanism linking inflammation to bladder pain across diverse models of IC/BPS, with TLR7 implicated in the Hunner-type subtype. Translating this insight into TLR-directed biomarkers and therapies will, however, require validation in larger clinical cohorts.

Bladder Outlet Obstruction (BOO)

BOO is the general term for any obstruction to voiding, characterized urodynamically by a reduced urinary flow rate together with an increased detrusor pressure [5]. Regardless of its underlying cause, BOO obstructs the bladder outflow tract between the bladder neck and the urethral meatus, either through mechanical compression or through increased resistance to urinary flow, and it characteristically produces LUTS that may be obstructive, irritative, or a combination of both [42]. BOO is common, having been estimated to affect approximately 21.8% of the global adult population, with a higher prevalence in men than in women (24.7% versus 18.9%) [4]. The most frequent cause is benign prostatic obstruction secondary to benign prostatic hyperplasia, whereas other etiologies include urethral stricture, dysfunctional voiding, neurogenic detrusor-sphincter dyssynergia, and primary bladder neck obstruction; in women, BOO most often arises iatrogenically after stress urinary incontinence surgery or from pelvic organ prolapse [42].

In the long term, BOO may lead to progressive and only partially reversible changes in bladder structure and function [42]. Experimentally, the obstructed bladder progresses through a series of distinct phases [43]. The sustained increase in outlet resistance reduces blood flow within the bladder wall, and the resulting cycles of ischemia and reperfusion generate free radicals that cause tissue injury and initiate an inflammatory phase. This is followed by a compensation phase, in which smooth muscle hypertrophy enables the bladder to generate the high intravesical pressures needed to overcome the obstruction, and, if the obstruction persists, by a decompensation phase characterized by tissue fibrosis and impaired, poorly reversible bladder function [44]. In a prolonged animal model of partial BOO, this sequence was reflected in an early inflammatory response with upregulation of transforming growth factor-β, connective tissue growth factor, and hypoxia-inducible factor-1α, followed by smooth muscle hypertrophy and a progressive increase in collagen deposition. At the same time, bladder capacity initially rose and then gave way to small-capacity, high-pressure bladders. The early appearance of these inflammatory and profibrotic mediators highlights the inflammatory phase as a potential window for therapeutic intervention [43].

Since the inflammatory phase initiates the structural cascade in BOO, the receptors that drive this early inflammation, including the TLRs, have attracted increasing attention. As pattern-recognition receptors of the innate immune system, TLRs detect both pathogen-derived molecules and damage-associated molecular patterns from injured cells, and their activation by these endogenous ligands can generate free radicals and contribute to ischemia-reperfusion injury. On this basis, TLR4 and TLR9, both with established roles in ischemia-reperfusion injury, were examined in an animal model of BOO comparing obstructed and sham-operated rats, with and without the α1-adrenergic antagonist tamsulosin [15]. In this model, obstructed bladders showed chronic inflammation with mononuclear infiltration but no acute inflammation, and obstruction increased the expression of both receptors, with TLR4 localized to the urothelium and detrusor smooth muscle and TLR9 confined to the urothelium. Obstruction also raised urinary IL-6, a cytokine linked to TLR activation, whereas IL-1β was unchanged. Tamsulosin did not alter TLR4 or TLR9 expression but reduced urinary IL-6 and IL-18 levels, suggesting a modest anti-inflammatory effect. α1-adrenergic receptors colocalized with the TLRs, providing an anatomical basis for crosstalk between the two systems. These findings implicate TLR4 and TLR9 in the bladder inflammation that constitutes the first phase of BOO and suggest that urinary IL-6 may serve as an adjunct biomarker of this phase, pend-

ing validation in humans [15]. The evidence is therefore preliminary and primarily associative, as the study documents only the upregulation and co-localization of TLR4 and TLR9 during the inflammatory phase. Still, it does not test, through genetic deletion or selective antagonism, whether these receptors causally drive obstruction-induced remodeling. This contrasts with IC/BPS and DBD, where causality has been probed directly. Accordingly, BOO is best regarded not as a well-established TLR-mediated disease model but as an important and underexplored area in which a potential TLR contribution has been raised and now requires confirmation in additional, mechanistically designed preclinical studies. In this context, the proposed value of urinary IL-6 as a biomarker of the inflammatory phase is appealing. Still, it is limited by its lack of specificity, since IL-6 is elevated in numerous inflammatory and infectious conditions of the lower urinary tract. Its utility would need to be established against appropriate controls before clinical application. The colocalization of α1-adrenergic receptors with TLR4 and TLR9 also raises the untested possibility of pharmacological synergy between α1-adrenergic blockade and TLR-directed strategies, warranting dedicated investigation.

Overall, the available evidence identifies the inflammatory phase as the pivotal early event that links outlet obstruction to subsequent bladder remodeling, with TLR4 and TLR9 emerging as candidate initiators of this process. The TLR system, therefore, represents both a potential target for halting the progression to fibrosis and, through readouts such as urinary IL-6, a possible source of biomarkers for the inflammatory phase of BOO. These conclusions nonetheless rest largely on a single animal study, and validation in further preclinical models and in patients will be needed before TLR-directed diagnostic or therapeutic strategies can enter clinical practice.

Diabetic Bladder Dysfunction (DBD)

DBD, historically termed diabetic cystopathy, is one of the most common urological complications of diabetes mellitus. It typically develops insidiously in middle-aged or elderly patients with long-standing, poorly controlled disease and spans a broad clinical spectrum rather than a single syndrome. In its classic form, damage to visceral afferent fibers in the bladder wall results in reduced sensation and contractility, increased bladder capacity, and increased post-void residual volume. Many patients, however, present earlier with OAB symptoms such as urgency, frequency, and nocturia, with or without incontinence, and bladder hypersensitivity with hypercontractility is in fact more common than hypocontractility [9]. Because lower urinary tract dysfunction in diabetes also involves the urethra and, in men, the prostate, its manifestations extend to underactive bladder, urinary incontinence, and aggravated symptoms of benign prostatic hyperplasia [45].

Diabetes is a disease of high and rapidly growing global prevalence, and bladder complications rank among its most frequent sequelae [24,45]. Lower urinary tract dysfunction is estimated to occur in around 80% of diabetic patients, while urinary bladder dysfunction specifically affects approximately half of them [45]. Earlier prevalence figures vary even more widely, in part because aging, benign prostatic hyperplasia, and concurrent neurological disorders make the specific diabetic contribution difficult to isolate [9]. The presence of diabetes is associated with both a greater likelihood and a greater severity of lower urinary tract symptoms [45]. The relationship between glycemic control and bladder outcomes, however, is not straightforward. Large follow-up data indicate that strict glucose control does not clearly reduce the risk of urinary incontinence, even though it lowers the risk of retinopathy, nephropathy, and neuropathy. At the same time, some studies have linked higher glycated hemoglobin levels to incident incontinence, leaving the role of glycemic control only partly resolved [24].

The pathogenesis of DBD is multifactorial, with alterations in detrusor muscle physiology, neuronal impairment, and urothelial dysfunction all contributing [9]. A widely cited framework for DBD is the temporal theory, which proposes two phases [9,45]. In the early phase, hyperglycemia-induced osmotic diuresis and polyuria increase bladder filling and stretch, producing compensatory bladder hypertrophy together with myogenic and neurogenic changes that present as an overactive, hypercontractile bladder. With time and the accumulation of toxic metabolites, bladder tissue decompensates, leading to the hypocontractile or atonic bladder of advanced disease [9]. Animal data support this progression, with streptozotocin models showing an early overactive phase that transitions toward underactivity over subsequent weeks [24]. At the level of the detrusor, diabetes enhances responses to muscarinic agonists, an effect attributed to increased muscarinic receptor density and greater smooth muscle sensitivity to calcium [9]. Chronic hyperglycemia also drives oxidative stress through activation of the polyol pathway and aldose reductase, protein kinase C signaling, and the formation of advanced glycation end products, which together damage smooth muscle and nerves [9,45]. Reduced nerve growth factor expression and impaired axonal transport contribute to the sensory neuropathy underlying bladder hyposensation. The urothelium, once viewed as a passive barrier, is now recognized as a sensory and signaling tissue whose dysfunction alters the release of mediators such as adenosine triphosphate, nitric oxide, and prostaglandins, thereby compromising barrier integrity [9]. Although polyuria and hyperglycemia-induced oxidative stress have long been regarded as primary drivers, neither factor alone fully accounts for DBD or its clinical progression, which has directed attention toward inflammatory mechanisms [14]. Activation of the innate immune system has emerged as a unifying mechanism in

the pathogenesis of diabetes and its complications, including DBD. Diabetes produces a systemic state of low-grade sterile inflammation, sometimes called meta-inflammation, in which pattern recognition receptors are engaged not by pathogens but by endogenous danger signals [24].

TLR4 is a central member of this receptor family. It is classically activated by bacterial lipopolysaccharide on immune cells and signals through the adaptor MyD88 to activate the NF-κB pathway, driving the production of reactive oxygen species and proinflammatory cytokines. Importantly, TLR4 is also expressed on non-immune cells, including bladder smooth muscle and urothelium, where endogenous damage-associated molecular patterns can activate it. HMGB1, an endogenous TLR4 ligand released from injured cells, is elevated in the circulation of patients with type 1 and type 2 diabetes and represents a likely driver of TLR4 activation in the diabetic bladder [14].

Direct evidence for a causal role of TLR4 in DBD comes from a streptozotocin model of type 1 diabetes. In that work, bladder expression of TLR4 and MyD88, and serum levels of HMGB1, were increased in diabetic animals, and *ex vivo* incubation of bladder tissue with recombinant HMGB1 increased TLR4 and MyD88 expression and enhanced contractile responses. Pharmacological inhibition of TLR4 with CLI-095 normalized the heightened contractile response of diabetic bladder strips, and genetic deletion of TLR4 protected mice from diabetes-induced bladder hypertrophy and hypercontractility despite comparable hyperglycemia. The upregulation of downstream mediators such as TRIF, IRAK, and the p65 subunit of nuclear factor kappa B reinforced the involvement of this pathway [14]. Taken together, these findings indicate that TLR4 activation is necessary and sufficient to mediate the bladder hypertrophy and hypercontractility of early DBD, positioning TLR4 as a candidate link between chronic hyperglycemia and the oxidative and inflammatory injury that follows. TLR4 does not act in isolation within the innate immune response of the bladder. The same diabetic danger signals also engage NLRP3 inflammasome, a sterile inflammation-inducing complex that has likewise been identified as a critical driver of DBD [24]. Activation of NLRP3 leads to the maturation and release of proinflammatory cytokines and to urothelial pyroptosis, a form of programmed cell death that compromises the urothelial barrier; the resulting release of further danger signals activates NLRP3 in neighboring cells in a feed-forward manner that may propel the bladder toward an underactive phenotype. Other inflammatory mediators, including tumor necrosis factor alpha, have also been implicated, and their inhibition improves bladder function in experimental diabetes [24,45]. Reflecting this convergent evidence, anti-TLR4 and anti-NLRP3 strategies are now discussed among the emerging, pathophysiology-targeted treatment approaches for diabetes-associated lower urinary tract dysfunction [45]. Collectively, these observations frame innate immune activation, with TLR4 at its core, as a promising therapeutic target upstream of the oxidative stress and structural remodeling that characterize DBD. An important limitation, however, is that this evidence pertains almost exclusively to the early, hypertrophic and hypercontractile phase of DBD. The causal studies that establish a role for TLR4 were all conducted in streptozotocin-induced models examined at relatively early time points, and the TLR4/HMGB1 and NLRP3 axes have been characterized chiefly as drivers of the compensated, overactive bladder. By contrast, the late, decompensated phase, marked by detrusor fibrosis and a hypocontractile or atonic bladder, remains poorly characterized with respect to TLR signaling. Whether sustained TLR4/NLRP3 activation and urothelial pyroptosis actively drive the transition to this fibrotic, hypocontractile stage, or whether the pathway is instead downregulated once decompensation is established, remains untested and represents a key unresolved question. A plausible yet hypothetical cascade links chronic hyperglycemia to the release of DAMPs such as HMGB1, engagement of TLR4, and downstream activation of the NLRP3 inflammasome, but the temporal dynamics of this axis over the full course of DBD await a dedicated longitudinal study.

Discussion

Summary of Current Evidence

Taken together, the studies reviewed here implicate TLR signaling, and TLR4 in particular, as a convergent mechanism linking sterile inflammation to bladder dysfunction across IC/BPS, BOO, and DBD. However, the supporting evidence differs markedly in strength among these conditions.

Core Limitations

The evidence base, however, remains uneven across the three conditions and, for BOO in particular, largely preliminary. TLR4 has been characterized most thoroughly in the context of bladder pain, above all in IC/BPS, where its contribution has been tested causally through genetic deletion and selective antagonism; by comparison, its role in disordered bladder function and voiding is far less defined, and for BOO the evidence remains limited and rests on a single preclinical study, so that the evidence for BOO should be regarded as preliminary and primarily associative rather than as establishing a well-defined TLR-mediated disease mechanism. Across all three conditions, primary studies that place a TLR at the center of the experimental design and directly test causality, rather than documenting expression or association, remain few. The most striking feature of the field is its near-exclusive preoccupation with a single receptor, having developed into something close to a TLR4 monoculture, leaving the contributions of other bladder-expressed receptors, such as TLR2, TLR5, TLR7, and TLR9, largely unexplored. Beyond these three disor-

ders, TLR4 has been examined only sporadically in other bladder conditions. Even the direction of its regulation may be context-dependent. In experimental radiation cystitis, for example, bladder irradiation downregulated urothelial TLR4 and IL-6 expression rather than inducing them [35], in contrast to the upregulation that characterizes IC/BPS, BOO, and DBD. A further and clinically important gap concerns disease stage, since existing work has concentrated almost entirely on the early, overactive and hypercontractile phase, particularly in OAB and DBD, leaving the role of TLR signaling in the later, fibrosis-associated, decompensated and hypocontractile bladder essentially unknown.

Challenges in Clinical Translation

Translating these preclinical findings into clinical therapy nonetheless faces several obstacles. The selective TLR4 antagonists used throughout the literature, principally TAK-242 (resatorvid) and its analog CLI-095, remain experimental tools, and no TLR4-directed small molecule has been approved for any indication. Because TLR4 is central to host antibacterial defense in the bladder, where it drives the cytokine response to uropathogens and limits bacterial invasion [22], systemic and sustained TLR4 blockade carries the theoretical liability of impairing innate immune surveillance and predisposing to urinary and systemic infection. This concern provides a strong rationale for localized, intravesical delivery, which could concentrate the drug at the urothelium while limiting systemic exposure; the demonstration that bladder-restricted TLR4 mediates pain and that locally administered TAK-242 is sufficient to relieve it supports the feasibility of such an approach [31]. The natural products and biological agents highlighted here face their own development hurdles, as plant-derived flavonoids such as baicalin are constrained by limited oral bioavailability and the need for standardization [27]. Similarly, mesenchymal stem cell-derived extracellular vesicles, although attractive as vehicles for anti-inflammatory cargo such as miR-9 [33], must overcome substantial challenges in large-scale manufacturing, quality control, targeting, and regulatory acceptance before clinical use.

Future Research Directions

Closing these gaps will require causal, mechanistically focused studies that extend beyond TLR4, span the full temporal course of each disorder, and are ultimately validated in larger clinical cohorts before TLR-directed biomarkers and therapies can be translated into clinical practice. Three priorities are especially tractable. First, the candidate TLR-related biomarkers identified so far, most notably the TLR4-stimulated inflammatory composite that tracks widespread pain in IC/BPS [29,30] and urinary IL-6 in BOO [15], should be validated prospectively in large, well-phenotyped clinical cohorts to establish their specificity and predictive value. Second, the causal evidence obtained to date has relied on global TLR knockout or systemic antagonism [13,14,16], so that tissue-specific (urothelial or detrusor) and conditional knockout models are needed to identify the cellular source of the pathogenic signal and to refine target definition. Third, because TLR4 operates in concert with downstream and parallel pathways, combination strategies, such as pairing TLR4 blockade with NLRP3 inflammasome inhibition [23,24] or with α1-adrenergic antagonism in the obstructed bladder [15], merit systematic evaluation. Addressing these priorities, while broadening attention to the bladder-expressed receptors that remain largely unexamined, offers the clearest path toward translating TLR biology into diagnostic and therapeutic advances for bladder dysfunction.

Conclusion

Beyond its established role in antibacterial defense, TLR signaling in the bladder is now recognized as a sensor of endogenous injury, engaged by DAMPs such as HMGB1 and coupled to NF-κB and the NLRP3 inflammasome. Across IC/BPS, BOO, and DBD, TLR4 emerges as a shared upstream node linking sterile inflammation to bladder pain, detrusor hypertrophy, and altered contractility, and its pharmacological inhibition attenuates these changes across chemically induced, autoimmune, and diabetic models. The strength of the evidence is nevertheless graded. Causality has been established in IC/BPS, where receptor deletion and selective antagonism attenuate bladder nociception, in one model without altering the accompanying inflammation or voiding dysfunction, and in DBD, where TLR4 deletion prevents bladder hypertrophy and hypercontractility despite comparable hyperglycemia, whereas the BOO literature remains associative. The field is constrained in three respects. Attention has centered almost entirely on TLR4; the experimental focus has rested on the early, overactive phase rather than the decompensated one; and no TLR-directed agent is in clinical use for any bladder disorder, the receptor's parallel role in host defense making sustained systemic blockade a particular liability. Closing these gaps will require causal studies extending across receptors, disease stages, and cell types, together with prospective validation of candidate biomarkers in adequately phenotyped patient cohorts.

Availability of Data and Materials

Not applicable.

Author Contributions

ÖT: Conceptualization, Investigation, Visualization, Writing-Original Draft. SE: Conceptualization, Investigation, Supervision, Writing-Review & Editing. Both authors gave final approval of the version to be published. Both authors have participated sufficiently in the work to take

public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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