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Toll-like receptors in kidney disease

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Abstract

Purpose of review—The innate immune system is our first line of disease against infection and injury, and responsible for initiating inflammatory and immune responses to resolve infections and repair injured tissues. This review focuses on the Toll-like receptors (TLRs) of the innate immune system and their role in recognizing infection and injury, and regulating inflammatory responses in the kidney.

Recent findings—There is increasing data to support a role for TLRs in immune complex-mediated glomerulonephritis. TLR7 has emerged as a key regulator of autoantibody production in murine lupus nephritis. In addition, studies have implicated TLR recognition of endogenous molecules released during cellular necrosis as critical regulators of sterile inflammation and injury. Tonic interactions between TLRs and environmental agonists derived from commensal microbes and endogenous sources may also influence autoimmune disease and inflammatory disorders affecting the kidney.

Conclusion—Future studies to decipher the contribution of TLRs and other innate immune receptors in the regulation of inflammation, immune responses, and injury in the kidney will pave the way for novel therapeutic interventions.

Keywords

autoimmunity; endogenous agonists; inflammation; innate immunity; sterile injury; Toll-like receptors

Introduction

Toll-like receptors (TLRs) are members of a growing family of innate immune receptors and the focus of this review. Innate immune receptors detect infection and regulate inflammatory and immune responses. This traditional view of innate immunity has been transformed by in-depth study over the past several years to reveal a much broader role for the innate immune system in regulating the interactions between the host and its environment.

For simplicity, we can divide the innate immune system's interaction with our environment into three classes (Fig. 1). The first is local recognition of infection and injury, in which local activation of innate immunity yields potent inflammatory responses and the initiation of immunity. The second class is systemic activation of innate immunity by infection or injury, which may indirectly affect the kidney through release of inflammatory mediators or the propagation of systemic diseases. The third class is homeostatic interactions between innate immune system and environmental stimuli. The innate immune system is constantly receiving input from our environment and these signals may influence self-nonsel discrimination

(allergic and autoimmune responses, and transplantation), as well as tissue healing and repair in response to injury.

Toll-like receptor recognition of agonists

Long-awaited breakthroughs in structural studies on TLRs have demonstrated the molecular details of how some agonists bind and activate TLRs [1–3]. Structures of the TLR2/TLR1 heterodimer with triacylated lipopeptide and TLR3 with double-stranded RNA demonstrate the precise nature of ligand-induced receptor dimerization that is required for activation [1,3]. Both the TLR3 and TLR2/1 agonists bind to specific sites and form a bridge between the receptors. This bridging interaction brings together the glycan-free surfaces of the TLRs, and the dimer is further stabilized by extensive intermolecular protein–protein interactions between the TLR molecules. The general model for TLR recognition of agonists that emerges from these studies is that the individual TLRs use diverse means of ligand binding and intermolecular interactions to form dimers that have similar quaternary structure, which properly aligns the cytoplasmic domains to initiate signal transduction.

The structure of the TLR4/myeloid differentiation factor 2 (MD-2) complex with eritoran, a synthetic antagonist of lipopolysaccharide (LPS), suggests that ligand-induced conformational changes may be an additional mechanism for ligand recognition [2]. In this structure, the four acyl chains of eritoran are tightly bound in the hydrophobic pocket of MD-2 and occupy greater than 90% of the pocket's surface. MD-2 is bound to TLR4, and there is no direct interaction between eritoran and TLR4. Because agonistic forms of LPS have up to six acyl chains, conformational changes in the MD-2 LPS complex are predicted to accommodate the additional acyl chains. It is possible that conformational differences between the MD-2/eritoran and MD-2/LPS complexes determine whether or not TLR4 dimerizes. Future structures with agonist forms of LPS should provide insight into the molecular mechanisms that induce TLR dimerization.

Local regulation of kidney disease by exogenous agonists of Toll-like receptors

Exogenous TLR agonists access the kidney through two major routes: direct infection and via circulatory system. The best studied system of direct infection is urinary tract infection and pyelonephritis. Multiple TLRs contribute to the host innate immune response to urinary tract infection by *Escherichia coli*, including TLR4, TLR5, and TLR11 [4]. These clever bugs also inject Toll-interleukin-1 receptor (TIR) domain containing proteins into host cells that interfere with TLR signaling and increase virulence [5]. Despite the convincing role of TLRs in mouse models of bacterial infection, less is known about the role for these receptors in human bacterial infections of the kidney. In fact, individuals with homozygous defects in MyD88 or interleukin-1 receptor-associated kinase 4 (IRAK4), essential signaling adaptors for TLR1, 2, 4, 5, 6, 7, 8, and 9 (Fig. 2), have no reported increase in urinary tract infections [6]. Remarkably, these individuals primarily suffered from increased susceptibility to a narrow range of pyogenic bacterial infections, most commonly *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and these infections were limited to childhood and early adolescence [6,7]. Little is known about the role of innate immunity to polyoma virus, which has become a substantial problem in renal transplantation. One recent study on mice [8] demonstrated that MyD88 contributed to the development of long-lasting antibody-mediated immunity to polyoma. A better understanding of the role of innate immunity in controlling polyoma virus infection may lead to novel strategies to increase natural immunity to this virus and preserve renal function in kidney transplant and immunosuppressed patients.

TLR agonists may also be circulating in the blood free or as a component of immune complexes, which are then deposited in the kidneys, frequently glomeruli, causing local activation of the innate immune system. This model is mostly theoretical and although supported by circumstantial evidence, we still have very little evidence to support the deposition of TLR agonists in the kidney and whether such deposited TLR agonists contribute to kidney disease. For immune complex-mediated glomerulonephritis, there is evidence that viral agonists of TLRs may contribute to the immune complex-mediated glomerulonephritis. TLR3 mRNA is upregulated in glomerular mesangial cells in hepatitis C virus (HCV)-associated membranoproliferative glomerulonephritis [9], and TLR7 polymorphisms have been linked to chronic HCV infection [10]. In addition, HCV core and NS3 proteins have been reported to activate macrophages through TLR2 [11]. Thus, TLRs may recognize HCV in immune complexes and this could aggravate glomerulonephritis. In addition to HCV-associated glomerulonephritis, TLR agonists may also be components of other infection-associated glomerulonephritides such as postinfection glomerulonephritis and IgA nephropathy. Thus, local recognition of TLR agonists in immune deposits may affect the inflammatory activity of immune complex-mediated glomerulonephritis.

The contribution of local vs. systemic TLR recognition to disease progression has been technically difficult to differentiate in mouse models, and the experimental approach that has been most frequently relied upon to make this distinction is the generation of bone marrow chimeras. This strategy helps to define the contribution of hematopoietic cells and nonhematopoietic cells, but falls short of defining cell-type-specific contributions. The recent generation of inducible MyD88-deficient mice provides a new tool to evaluate the cell-type-specific responses of TLRs using the Cre-LoxP system [12•] and this should provide even more details about the precise role of intrinsic TLRs in the kidney.

Local regulation of kidney disease by endogenous agonists of Toll-like receptors

Sterile injury provokes inflammation and this inflammatory response has several physiologic and pathologic outcomes. The inflammatory response to injury is critical for wound healing and repair, and defense against opportunistic pathogens. However, the resulting inflammation may also lead to excessive tissue destruction and impaired organ function. Rodent models of toxin and ischemia-induced renal injury have demonstrated that inflammation contributes to the development of renal failure [13,14]. What are the receptors and ligands that trigger inflammation in these settings of sterile kidney injury? Recent studies implicate TLR2 and TLR4 as key regulators of inflammation in ischemia/reperfusion injury (IRI), but the results are complex. TLR2-deficient mice were protected from IRI [15,16••] and using bone marrow chimeras, Leemans *et al.* [15] found that the TLR2 effect was mediated by nonhematopoietic cells. Shigeoka *et al.* [16••] tested MyD88-deficient mice, which were also protected from IRI, but surprisingly, the MyD88 effect was substantially less than that seen for TLR2-deficient mice. In contrast, Wu *et al.* [17••] demonstrate substantial protection from IRI in TLR4-deficient mice and MyD88-deficient mice. Yet another study by Pulsikens *et al.* [18••] concludes that TLR4-deficient mice are protected from IRI, whereas MyD88-deficient mice and TIR domain-containing adapter-inducing IFN- β (TRIF)-deficient mice are not. This group also found that the TLR4 effect is mediated by nonhematopoietic cells [18••]. In summary, these studies have found that TLR2 and TLR4 contribute to inflammation and renal insufficiency in IRI, and when tested these effects are mediated by nonhematopoietic cells. However, the studies disagree about the contribution of MyD88, the primary signaling adaptor for TLRs (except TLR3, Fig. 2). Future studies will be needed to clarify the roles of TLRs and their signaling pathways in IRI. TLR4 expressed in nonhematopoietic cells has also been

demonstrated to contribute to the inflammatory response and kidney injury secondary to cisplatin [19].

A large number of molecules have been implicated as endogenous agonists of TLRs (Table 1) [20–36], and some of these could possibly mediate IRI. Wu *et al.* [17••] looked for upregulation of candidate TLR4 agonists and discovered that high mobility group box 1 (HMGB1), biglycan, and hyaluronan were increased in the ischemic kidney. The ensuing model is that IRI produces endogenous TLR4 agonists that activate TLR4 expressed by resident kidney cells, leading to inflammation and renal dysfunction. Unfortunately, much of the data concerning endogenous agonists of TLRs are plagued with issues of contamination [24,37,38]. HMGB1 has been ascribed multiple mechanisms for activating TLRs, which include direct binding to TLR2 and TLR4 [30,39], binding cellular DNA to promote endocytosis by receptor for advanced glycation endproducts (RAGE) and TLR9 activation [40], and promoting CD14-dependent TLR4 recognition of LPS [41]. Recombinant biglycan activates TLR2 and TLR4 *in vitro*, and biglycan-deficient mice are protected from sepsis induced by LPS or yeast cell wall [42]. Biglycan has also been shown to bind tumor necrosis factor (TNF) and transforming growth factor- β (TGF- β) and this activity may be relevant *in vivo*. Future studies should elucidate the molecular mechanisms of TLR2 and TLR4 activation during ischemic or toxic kidney injury and the role of endogenous TLR agonist *in vivo*.

Systemic activation of Toll-like receptors and kidney disease

Systemic activation of the innate immune can affect the kidney in multiple ways; we will focus on two pathways. The first is innate immune system activation of B lymphocytes, leading to the production of autoantibodies and immune complex-mediated glomerulonephritis. The second is the exacerbation of preexisting kidney disease by TLR agonists. Most of these data stem from mouse models, which may provide some insight into human disease.

Mouse models and human genetic studies have provided additional evidence that innate immune system regulates autoantibody production, and in particular lupus autoantibodies and rheumatoid factor. The recent advances in our understanding of the roles of TLRs in systemic lupus erythematosus (SLE) have revealed the close link between the nature of autoantigens in lupus and the ligand specificity of the TLRs, namely TLR7 and TLR9. These findings also unveiled an imperfection in the innate immune system – innate discrimination of host from pathogen is not absolute. This is true in particular for the nucleic acid TLR agonists. Thus, dsRNA (TLR3), ssRNA (TLR7 and TLR8), and CpG DNA (TLR9) are not chemical structures that are unique to microbes. The ability to discriminate host nucleic acids from microbial nucleic acids appears to rely on a combination of properties, including sequence motifs, tertiary structure, chemical modifications (i.e. cytosine methylation), and the endosomal localization of these TLRs. TLR recognition of host nucleic acids may be a promiscuity that has been tolerated throughout evolution, because the selective advantage of pathogen detection outweighs the risk of autoantibody production.

Rheumatoid factor is a common component of cryoglobulins in mixed cryoglobulinemia, which can cause membranoproliferative glomerulonephritis and is a problematic manifestation of HCV infection. Elegant studies on a mouse model demonstrated that rheumatoid factor-producing B cells were elicited in extrafollicular sites by immune complexes that contain IgG and chromatin through a mechanism requiring surface IgM, TLR7, TLR9, and MyD88 [43••]. This generation of autoreactive rheumatoid factor-producing B cells did not require T-cell-specific signals [43••]. Thus, costimulation of autoreactive B cells by autoantigens, which are TLR agonists or associated with TLR agonists, may be a common mechanism for autoantibody production.

The role of innate immunity in lupus nephritis has been recently reviewed [44]. Both TLR7 and TLR9 have been implicated in autoantibody production in lupus, but recent studies on a murine lupus model (the MRL/Mp^{lpr/lpr} mouse) suggest that these receptors may have opposing roles [45]. TLR7-deficient mice did not produce anti-Smith or antiribonucleoprotein (RNP) antibodies and they were protected from lupus nephritis. In contrast, the TLR9-deficient mice did not make anti-DNA antibodies, but they had accelerated disease. Duplication of the TLR7 gene in Yaa mice was responsible for their lupus phenotype [46,47] and TLR7 was also required for the mouse model of pristane-induced lupus nephritis [48,49]. Thus, there is substantial evidence that copies of TLR7 or excess production of endogenous TLR7 agonists contribute to lupus nephritis in the mouse. Overexpression of TLR4 in mice also resulted in autoantibodies and lupus-like nephritis in C57BL/6 mice [50]. In addition, deletion of single Ig interleukin-1 receptor-related molecule (SIGIRR), a negative regulator of TLR signaling (Fig. 2), exacerbated lupus in mice [51]. These studies support a role for dysregulation of TLRs (or their agonists) in lupus and have uncovered a surprising protective role for TLR9.

The models of nephrotoxic serum-induced glomerulonephritis, membranoproliferative glomerulonephritis, in the mouse suggest that systemic activation of the innate immune system can increase inflammation and injury within the kidney. Passive transfer of antiglomerular basement membrane (GBM) antibodies induced only mild disease, which is markedly exacerbated by coadministration of TLR agonists [52]. Similarly, TLR9 agonists augmented glomerular inflammation in a mouse model of membranoproliferative glomerulonephritis [53]. These studies suggest that systemic activation of the innate immune system can aggravate preexisting kidney disease.

Homeostatic interactions between Toll-like receptors and environmental stimuli

The interaction between host and commensals is another area that has emerged in recent years as an important aspect of innate immunity. A seminal study [54] demonstrated that TLR-dependent sensing of commensal bacteria in the gut is critical for epithelial repair to toxic injury induced by dextran sodium sulfate. This study demonstrated that TLRs can sense commensal bacteria and use this as a signal to initiate wound healing and repair. More recent studies have illuminated the continuous interaction between our innate immune system and the environment. Mice that are deficient in TNFAIP3 (Fig. 2), a key negative regulator of TLR signaling, develop a spontaneous autoinflammatory disorder that involves multiple organs, including the kidney, and results in marked cachexia and early death [55]. This autoinflammatory disorder is dependent on MyD88 and commensal bacteria [56•], suggesting that tonic recognition of commensals by TLRs is kept in check by negative regulatory mechanisms. Human polymorphisms in TNFAIP3 have also been associated with SLE [57]. Interestingly, the increased anti-dsDNA antibody titer in TLR4-overexpressing mice was dependent on commensal flora [50]. In humans, MyD88 and IRAK4 are required for the efficient removal of autoreactive B cells [58••]. Finally, the development of diabetes in the nonobese diabetic (NOD) mouse was also influenced by MyD88 [59]. Thus, the development of autoantibodies and autoimmune diseases may in part be influenced by homeostatic interactions between TLRs and our environment, in particular commensal microbes and endogenous agonists. It is interesting to speculate that homeostatic interactions between TLRs and our environment are continuously regulating our inflammatory rheostat and self-nonsel discrimination.

Beyond Toll-like receptors

Microbial infections are also recognized by additional families of innate immune receptors, which are increasingly recognized as important components of the innate immune response.

These families include the NOD-like receptors (NLRs) and the retinoic acid-inducible gene (RIG)-like helicases (RLHs) [60,61]. These families of receptors primarily detect microbial products that gain access to the cytosol, or alterations in host cell, such as the efflux of potassium. Importantly, extracellular ATP, uric acid crystals, and calcium phosphate crystals have been shown to activate the caspase-1 inflammasome and interleukin-1B and interleukin-18 secretion through an NLR-dependent pathway [62,63]. Future studies concerning these families of innate immune receptors should provide additional insight into the role of the innate immune system in recognizing infection and injury and regulating inflammation in kidney diseases.

Conclusion

These recent studies underscore the importance of the innate immune system in kidney disease. A more detailed understanding of the role of the innate immune system in sterile injury will help to clarify the receptors, signaling pathways, and agonists that regulate this response. There are also potential roles for modulating the innate immune system to promote the clearance of unwanted infections, such as polyoma virus infection, and to modulate tissue repair due to injury. Finally, we are now beginning to appreciate the existence of tonic signaling through TLRs via ligands that emanate from commensal bacteria or endogenous molecules, and that dysregulation of this homeostasis can influence inflammatory and autoimmune diseases. Future studies to decipher the contribution of TLRs and other innate immune receptors in the regulation of inflammation, immune responses, and injury in the kidney will pave the way for novel therapeutic interventions.

Acknowledgments

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Figure 1. Toll-like receptor regulation of kidney disease

Toll-like receptors (TLRs) can influence kidney disease through multiple mechanisms. Local activation of TLRs by infectious agents, planted agonists (i.e. in the form of immune complexes) or the generation of endogenous agonists in the setting of injury. Systemic activation of TLRs can lead to autoantibody production, such as rheumatoid factor or lupus autoantibodies, which can be deposited in the kidney. Systemic activation can also produce inflammatory mediators that can influence preexisting disease within the kidney. Homeostatic interactions between TLRs and our environment, commensal microbes and endogenous agonists, provide tonic stimulation that influences self-nonsel self discrimination and basal inflammatory states.

Figure 2. Toll-like receptor signaling module

Toll-like receptors (TLRs) signal through proximal adaptors. TLR2 (+1 or 6), TLR4, TLR5, TLR7, TLR8, and TLR9 signal through MyD88 and the interleukin-1 receptor-associated kinase (IRAK) complex to activate nuclear factor- κ B (NF- κ B) and mitogen-activated protein (MAP) kinase pathways, leading to the expression of a broad set of genes involved in inflammatory responses. For TLR2 and TLR4, the Toll-interleukin-1 receptor (TIR) domain-containing adaptor protein (TIRAP) is required for efficient and complete activation of this MyD88-dependent pathway. TLR7, TLR8, and TLR9 also activate IRF7 in a MyD88-dependent fashion leading to the expression of interferon- β (IFN- β) and type I IFN-associated genes. TLR3 signals through TIR domain-containing adapter-inducing IFN- β (TRIF), which activates the type I IFN response through IRF3 and also can activate NF- κ B and MAP kinase pathways through receptor-interacting protein 1 (RIP1). TLR4 also engages the TRIF pathway and this is facilitated by TRIF-related adaptor molecule (TRAM). The TLRs that can activate

type I IFN responses are shaded gray. Several negative regulator of TLR signaling are shown in black. Single Ig interleukin-1 receptor-related molecule (SIGIRR) is a TIR domain containing cell surface molecule that is believed to compete with TLRs for MyD88. IRAKM inhibits activation of the IRAK complex. TNFAIP3 inactivates TRAF6, blocking activation of NF- κ B and MAP kinases. Activating transcription factor 3 (ATF3) binds to the promoters of some TLR-regulated genes and alters chromatin structure to affect gene expression.

Table 1

Toll-like receptors and agonists

Receptor	Microbial agonists	Endogenous agonists	Synthetic agonists	References
TLR2 (+1 or 6)	Lipoproteins, peptidoglycan ^a , lipoteichoic acids ^a , glycosylphosphatidylinositol anchors, lysophosphatidylserine, lipophosphoglycan, lipoarabinomannan, lipopolysaccharide, zymosan, viruses (measles, herpes simplex, varicella zoster, cytomegalovirus, lymphocytic choriomeningitis, hepatitis C)	Hsp60 ^b , Hsp70 ^b , gp96 ^b , biglycan, hyaluronan, HMGB1 ^b , uric acid ^b , minimally modified LDL	diacylated and triacylated lipopeptides	[20–26]
TLR3	dsRNA (>40–50 bp)	mRNA	Poly-I,C	[27,28]
TLR4	Lipopolysaccharide, viruses (respiratory syncytial, mouse mammary tumor, and Coxsackie virus)	Hsp60 ^b , Hsp70 ^b , gp96 ^b , Hsp22, biglycan, uric acid ^b , hyaluronan, fibronectin, fibrinogen, heparan sulfate, surfactant protein A, HMGB1, β -defensin, Tamm-Horsfall protein	Monophosphoryl A	[24–26,29–31]
TLR5	Flagellin			[32]
TLR7	ssRNA	ssRNA	Imidazoquinolines, synthetic ribonucleic acids	
TLR8	ssRNA	ssRNA	Imidazoquinolines, Synthetic ribonucleic acids	
TLR9	CpG DNA, hemozoin	CpG DNA	CpG oligonucleotides	[33,34]
TLR10 ^c				
TLR11 ^c	Profilin			[35]
TLR12 ^c				
TLR13 ^c				

HMGB1, high mobility group box 1; LDL, low-density lipoprotein; TLR, Toll-like receptor.

^aPreparations of lipoteichoic acids and peptidoglycans may contain lipopeptides, which are the authentic TLR2 agonist.

^bSome studies show conflicting results, implicating contaminants as active components, or signaling through other receptors.

^cTLR10, which is predicted to pair with TLR2, is a pseudogene in mice. TLR11 is a pseudogene in humans, and TLR12 and TLR13 are not present in humans. Additional TLRs are present in other vertebrates, which do not have obvious orthologs in humans or mice [36].