Toll-like receptors: sensing and reacting to diabetic injury in the kidney

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ABSTRACT

Accumulating evidence indicates that immunologic and inflammatory elements play an important role in initiating and orchestrating the development of diabetic nephropathy (DN), but until recently, the identity of specific innate immune pattern recognition receptors or sensors that recognize diverse diabetic ‘danger signals’ to trigger the proinflammatory cascade during DN remains unknown. Toll-like receptors (TLRs) are an emerging family of receptors that recognize pathogen-associated molecular patterns as well as damage-associated molecular patterns to promote the activation of leukocytes and intrinsic renal cells in non-immune kidney disease. Recent data from in vitro and in vivo studies have highlighted the critical role of TLRs, mainly TLR2 and TLR4, in the pathogenesis of DN. This review focuses on emerging findings elucidating how TLR signaling could sense and react to the metabolic stress and endogenous ligands activated by the diabetic state, thereby initiating and perpetuating renal inflammation and fibrogenesis in diabetic kidney disease. Novel strategies potentially targeting TLR signaling that could have therapeutic implications in DN are also discussed.

Keywords: diabetic nephropathy, fibrosis, inflammation, toll-like receptor

INTRODUCTION

Diabetic nephropathy (DN) has been increasingly recognized to comprise a heavy inflammatory element triggered by metabolic disorders, protein overload and hemodynamic abnormalities [1–4]. In renal biopsies of human DN, persistent inflammation has been demonstrated by large clusters of neutrophils and macrophages infiltrating into the interstitial space, and the number of inflammatory cells were closely related to renal function at the time of the biopsy [5]. Moreover, the activation of NF-κB and certain proinflammatory chemokines/cytokines in tubular epithelial cells were correlated with the magnitude of the proteinuria and interstitial cell infiltration [6]. A complex array of tightly regulated events including various proinflammatory molecules (IL-1, IL-6, IL-8, IL-18 and TNF-α) [7, 8], multiple inflammatory signaling pathways [mitogen-activated protein kinases (MAPK), signal transducer and activator of transcription (STAT) and protein kinase C (PKC)] [9–11], renal intrinsic cells and diverse immune cell (macrophages, T cells and neutrophils) [12–15] interaction likely orchestrate the initiation and development of DN. Toll-like receptors (TLRs) are a conserved family of pattern recognition receptors that play a fundamental role in the innate immune system by triggering proinflammatory signaling pathways in response to microbial pathogens [16]. An extended family that includes 11 human and 13 mouse TLRs has been identified. Given the recent recognition that (i) TLRs are activated by endogenous ligands of nonmicrobial origin, namely damage-associated molecular patterns (DAMPs), which are involved in noninfectious inflammatory conditions [17], (ii) TLR expression on intrinsic renal cells and leukocytes has been reported to contribute to various acute and chronic kidney diseases (CKD) [18] and (iii) overexpression of TLR2 and TLR4 in monocytes is positively correlated with hemoglobin A1c (HbA1c) levels and homeostasis model assessment–insulin resistance (HOMA-IR) in T1DM and T2DM patients [19–21], investigation into the critical role of TLRs may unravel how the ‘metabolic danger’ sensed by the kidney could initiate the immunological cascade that culminates in DN. Herein, we review recent progress in this emerging field and highlight some novel strategies potentially targeting TLR signaling that could have therapeutic implications.

TLR SIGNALING PROMOTES RENAL INFLAMMATION AND FIBROSIS

TLRs belong to the ‘Interleukin-1 Receptor/Toll-Like Receptor Superfamily’, which have the same cytoplasmic domain as the TIR (Toll-IL-1 receptor) domain and share a similar pathway.
of activation. When activated, TLRs recruit different adapter molecules, and then initiate diverse downstream signaling cascades including MyD88-dependent and -independent pathways, resulting in engagement of transcription factors NF-κB and IRF-3, respectively, and the production of downstream proinflammatory cytokines and chemokines (Figure 1) [16, 17].

TLRs are expressed in various cell types in the kidney, including dendritic cells [22, 23], lymphocytes [24], macrophages [19–21] as well as intrinsic renal cells such as podocytes [25], mesangial cells [26, 27], endothelial cells [28–30] and tubular epithelial cells [31]. In addition to inflammatory and autoimmune kidney disease, cell-type-specific activation of TLR signaling, mainly TLR2 and/or TLR4, is also involved in the intertwining events of renal inflammation, leukocytes infiltration and progressive fibrosis in non-immune kidney disease, such as ischemia/reperfusion (I/R) injury [32, 33], obstructive uropathy [34–36], tubulointerstitial nephritis [37], nephrotoxicity [38–40] and DN [41, 42]. Several studies using TLR4−/− and TLR2−/− mice have demonstrated that blockade of TLR2 or TLR4 may have protective effects in models of acute kidney injury (AKI) and CKD. For example, Zhang et al. [38] demonstrated that TLR4 expressed in parenchymal renal cells rather than myeloid cells mediates renal effects in models of acute kidney injury (AKI) and CKD. For example, Zhang et al. [38] demonstrated that TLR4 expressed in parenchymal renal cells rather than myeloid cells mediates renal inflammation and injury in cisplatin-induced AKI, and TLR4−/− mice developed significantly lower levels of cytokines in serum, kidney and urine as well as less histologic damage compared with wild-type mice. Leemans et al. [43] used both TLR2−/− mice and antisense oligonucleotide to show that blockade of TLR2 has a protective effect on renal I/R injury. While in the study of renal fibrosis, TLR4−/− mice subjected to unilateral ureteral obstruction displayed less tubular fibrosis without affecting myofibroblast accumulation [34].

Among the TLRs, TLR4 plays a major role in renal inflammation during ischemia/reperfusion injury via MyD88-dependent and -independent pathways [32, 33]. The endothelial TLR4 could rapidly respond to I/R injury and activate interferon-γ and TNF-α, which then induce tubular cell to release endogenous TLR4 ligands, such as HMGB1, into the extracellular space. These TLR4 ligands not only indirectly facilitate macrophages to transmigrate into the interstitium by inducing endothelial expression of adhesion molecules, but also directly activate TLR4 on tubular cells and to a lesser degree on macrophages to induce inflammatory cytokines such as IL-6, resulting in the amplification of tubulointerstitial inflammation and exacerbation of kidney injury [28, 44]. On the other hand, TLR2 on intrinsic renal cells also plays an adjuvant role in renal I/R inflammation via MyD88-dependent and independent Rac-1/PI3K/Akt activation [43], while TLR4 on resident renal stem/progenitor cells may be associated with the repair processes after injury [45, 46].

TLR signaling may contribute to renal fibrosis through diverse cell-type specific mechanisms as shown recently in unilateral ureteral obstruction models. TLR4 promotes tubulointerstitial fibrosis via TGF-β-smad independent mechanisms by (i) enhancing the susceptibility of tubular cells and myofibroblasts toward TGF-β signaling, which is associated with the inhibition
promote in (Table 1) [52, 61]. Besides, fibrinogen, another endogenous ligand for TLRs, may deposit in the kidney and aggravate renal fibrosis by triggering resident fibroblast proliferation via TLR2/TLR4-MyD88/NF-κB-dependent pathway [35]. Moreover, Th2 inflammatory cytokines associated with macrophage recruitment may also be involved in renal fibrosis in a MyD88-dependent manner [48]. However, the role of TLR2 in renal fibrosis is controversial. Although there is a reduction of interstitial fibroblast at later stages, overall renal fibrosis was not affected by TLR2 [49].

### Table 1. Proposed endogenous ligands for TLR2/4 in diabetic-associated disorders

<table>
<thead>
<tr>
<th>Endogenous ligands</th>
<th>TLRs</th>
<th>Refs</th>
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<tbody>
<tr>
<td>Cell derived</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSP90, HSP70, GP96</td>
<td>TLR2/TLR4</td>
<td>[53, 62]</td>
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<tr>
<td>HMGBl</td>
<td>TLR2/TLR4</td>
<td>[63–66]</td>
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<tr>
<td>S100A 8/14</td>
<td>TLR4</td>
<td>[56, 67]</td>
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<tr>
<td>ECM derived</td>
<td></td>
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<tr>
<td>Fibrinogen</td>
<td>TLR4</td>
<td>[25, 57]</td>
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<tr>
<td>Surfactant protein A</td>
<td>TLR4</td>
<td>[58]</td>
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<tr>
<td>Heparan sulfate</td>
<td>TLR4</td>
<td>[59]</td>
</tr>
<tr>
<td>Hyaluronan</td>
<td>TLR2/4</td>
<td>[68]</td>
</tr>
<tr>
<td>β-Defensin</td>
<td>TLR4</td>
<td>[69, 70]</td>
</tr>
<tr>
<td>Extra domain A of fibroactin</td>
<td>TLR4</td>
<td>[71]</td>
</tr>
<tr>
<td>Biglycan</td>
<td>TLR2/4</td>
<td>[72, 73]</td>
</tr>
<tr>
<td>Tenasin-C</td>
<td>TLR4</td>
<td>[74]</td>
</tr>
<tr>
<td>Versican</td>
<td>TLR2</td>
<td>[75]</td>
</tr>
<tr>
<td>Others</td>
<td></td>
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<tr>
<td>mmLDL</td>
<td>TLR4</td>
<td>[76, 77]</td>
</tr>
<tr>
<td>OxLDL</td>
<td>TLR4</td>
<td>[78, 79]</td>
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<tr>
<td>AGE-LDL</td>
<td>TLR4</td>
<td>[80]</td>
</tr>
<tr>
<td>ω-(2-carboxyethyl)pyrrole (CEP)</td>
<td>TLR2</td>
<td>[81]</td>
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<tr>
<td>FFAs</td>
<td>TLR2/4</td>
<td>[82–84]</td>
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<tr>
<td>Fetuin-A</td>
<td>TLR4</td>
<td>[85]</td>
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<tr>
<td>Angiotensin II</td>
<td>TLR4</td>
<td>[26, 86]</td>
</tr>
<tr>
<td>β-Amyloid</td>
<td>TLR2</td>
<td>[87]</td>
</tr>
<tr>
<td>Serum amyloid A</td>
<td>TLR2/4</td>
<td>[88, 89]</td>
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The ‘danger’ theory by Matzinger [50] proposed that endogenous molecules, namely damage/danger-associated molecular patterns (DAMPs), can activate the immune system in a fashion analogous to pathogen-associated molecular patterns (PAMPs). The identification of endogenous ligands for TLRs has greatly extended our knowledge of the pathogenic role of TLRs in various noninfectious inflammatory conditions such as diabetes, atherosclerosis and chronic kidney disease [19–21, 51, 52]. Proteins in response to cell stress and injury such as HMGBl, heat shock proteins [53–55], S100 protein family [56], as well as degradation components of ECM remodeling such as fibrinogen [25, 57], surfactant protein-A purified native protein [58], heparan sulfate [59] and hyaluronan [60], have been shown to promote inflammatory response through binding to TLR2 and/or TLR4 via both intracellular and extracellular mechanisms (Table 1) [52, 61].

In addition to the damage-associated endogenous ligands mentioned above, emerging evidence showed that TLRs might be the molecular link between inflammation and metabolic syndrome associated disorders such as hyperglycemia, dyslipidemia, hyperuricemia and hemodynamic abnormalities: (i) Hyperglycemia: In vitro studies showed that high glucose (HG) induced TLR2 and TLR4 expression via PKC and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation, and knocking down of TLR2 and TLR4 significantly downregulated HG-induced NF-κB activation [90]. One of the mechanisms involved in HG-induced TLR activation in tubular epithelial cells could be the secretion of HMGBl, which is a nuclear DNA-binding protein that regulates transcription and promotes inflammatory response by binding to TLR2 and TLR4 [63–66]. (ii) Dyslipidemia: Because saturated FFAs are essential components for LPS to activate TLR signaling, it is postulated that FFAs modulate insulin resistance via TLRs. In vitro, FFAs could activate TLR2/4 NF-κB signaling in macrophages [82], muscle cells [91], podocytes [83] and vascular endothelial cells [84], which is closely associated with the inflammatory mechanisms of insulin resistance. Fetuin-A could act as an endogenous ligand of TLR4 to promote FFAs-induced insulin resistance [85]. Besides, FFAs also augment HG-induced proinflammatory effects in macrophages, adipocytes and podocytes via enhancing the expression of TLRs [83, 92], and S100A8 [67]. In vivo, TLR4 deficiency may protect against diet-induced obesity and insulin resistance via inhibition IkB kinase and c-Jun NH2-terminal kinase pathway [93, 94]. Furthermore, atherogenic lipoproteins such as minimally modified low-density lipoprotein, mmLDL, minimally modified low-density lipoprotein, AGE-LDL, advanced glycation end products-modified LDL, oxidized low-density lipoprotein [78, 79] trigger inflammation in monocytes via interaction with TLR4/TLR2, while advanced glycation end products-modified LDL can induce IL-6 and IL-8 production via the TLR2/4-MyD88-dependent pathway in tubular epithelial cells [80]. In addition, serum amyloid A in dysfunctional HDL may promote inflammatory cytokine production and endothelial dysfunction in CKD via TLR2 signaling [88, 95]. (iii) Uric acid: UA induced endothelial cells to release nuclear HMGBl, which then activated NF-κB activity and angiopoietin-2 expression via TLR4 in an autocrine/paracrine manner [96]. In vivo, TLR2/4-deficient mice were protected from monosodium urate monohydrate crystal-induced inflammation or tubulointerstitial nephritis [96]. (iv) Hemodynamic abnormalities: Angiotensin II (Ang II) promotes TLR4 expression by increasing AP-1-dependent TLR4 gene transcription in mouse mesangial cells [97]. Oxidative stress induces the formation of an end product of lipid oxidation, ω-(2-carboxyethyl)pyrrole (CEP),...
which may promote angiogenesis and endothelial migration through TLR2 signaling in a MyD88-dependent manner [81].

Therefore, the metabolic substrates associated with diabetes may directly interact with TLRs or indirectly promote the production of TLR endogenous ligands which then trigger the downstream events and the development of diabetes and diabetic complications. Further investigation into the mechanisms of TLR signaling activation in the diabetic state will facilitate the identification of more endogenous ligands for TLRs, which could have implications for targeting TLR signaling.

**TLRs in DN**

**The role of TLR4**

We recently observed that tubular TLR4 is the main mediator of DN. There are three levels of evidence: (i) in human DN, TLR4 but not TLR2 was highly expressed in the renal tubules, which correlated positively with interstitial macrophage infiltration and HbA1c levels, and negatively with estimated GFR at the time of biopsy. Tubular expression of HMGB1, a known TLR4 endogenous ligand activated by the diabetic state [21], was also increased in DN biopsies; (ii) in cultured human PTECs, HG induced TLR4 but not TLR2 expression via PKC activation, resulting in upregulation of IL-6 and CCL-2 expression via inhibitory kappa B (IkB)/NF-κB activation. Molecular silencing of TLR4 in PTECs with siRNA attenuated HG-induced IkB/NF-κB activation, the associated downstream IL-6 and CCL-2 synthesis and impaired the ability of PBMC/U937 mononuclear cell transmigration induced by HG-treated PTEC conditioned media; (iii) STZ-induced uninephrectomized TLR4−/− mice displayed significantly reduced albuminuria, renal dysfunction, renal cortical NF-κB activation, tubular CCL-2 expression and interstitial macrophage infiltrates than wild-type animals. A similar beneficial effect was also observed in STZ-induced eNOSKO mice, a model of advanced DN [100], treated with a systemic TLR4 inhibitor CRX-526 versus untreated mice [101]. CRX-526 could reduce albuminuria by suppressing renal inflammation and interstitial macrophage infiltration through downregulation of inflammatory chemokines CCL-2, CCL-5 and osteopontin, and attenuate renal fibrosis through the reduction of collagen deposition. These effects are likely mediated through the inhibition of TGF-β overexpression and NF-κB activation.

In addition to tubulointerstitial injury, TLR4 has been shown to have an active role in the development of albuminuria and glomerular damages. In animal models of glomerulonephrosis [102] and mesangio proliferative glomerulonephritis [25], podocyte TLR4 activation could induce proinflammatory cytokines and accelerate podocyte injury. In vitro studies have shown that diabetic substrates such as FFAs, Ang II and HG could upregulate TLR4 signaling in mouse glomerular endothelial cells, podocytes or mesangial cells [26, 27, 29, 86]. Recently, the role of TLR4 in diabetic glomerulopathy was further evidenced in two models of T2DN: (i) in a model of HFD combined with STZ-induced diabetes, activation of the TLR4/TRIF-dependent pathway and TLR4 endogenous ligand S100A8 contributed to the exacerbation of DN by hyperlipidemia. TLR4-deficient mice showed less albuminuria, mesangial expansion, infiltration of macrophages and proinflammatory and extracellular matrix-associated gene expression in glomeruli [67]; (ii) in db/db mice, treatment with a non-specific TLR inhibitor GIT27 not only decreased urinary albumin excretion, glomerulosclerosis and proinflammatory/pro-fibrotic cytokines IL-2, TNF-α PAI-1 and TGF-β synthesis, but also improved tissue lipid metabolism, insulin resistance and oxidative stress compared with the control group. In cultured podocytes and adipocytes, the beneficial effect of GIT27 was mediated through inhibition of HG with FFA-induced TLR4 upregulation and NF-κB, Nox4 and TNF-α synthesis [83]. However, due to the limitation of T2DN animal models used in these studies, the role of TLR4 in tubulointerstitial fibrosis in T2DN remains unknown. Overall, these results further highlight the important role of TLR4 signaling, as a kidney sensor for ‘metabolic danger’, in triggering the progress of T1DN and T2DN via both MyD88-dependent and -independent pathways.

**The role of TLR2**

The contribution of TLR2 to DN has been investigated by recent studies. In STZ-induced diabetic rats, the expression of TLR2 was significantly upregulated within both the glomeruli and tubulointerstitium, which was associated with increased renal expression of myeloid differentiation factor MyD88 and CCL-2, the activation of NF-κB, and infiltration of macrophages. The endogenous ligands for TLR4, HSP70 and HMGB1, were also activated. These findings were mirrored in a rat tubular cell line (NRK-52E cells) in which HG induced the expression of TLR2 mRNA. Furthermore, in a STZ diabetic TLR2−/− mouse model, diabetes induced the expression of TLR2/MyD88 signaling in macrophages, and mice deficient of TLR2 showed less albuminuria, decreased kidney nephrin, podocin and podocyte number and increased TGF-β and laminin compared with WT mice, which indicated that TLR2 could prompt diabetic inflammation and kidney injury in incipient DN [103]. More recently, the precise role of TLR2 in mediating inflammation was depicted in a human renal proximal tubular cell line (HK-2 cells), in which 11.2 mM glucose (the diagnostical threshold for diabetes mellitus) maximally increased TLR2 and TLR4 expression, HMGB1 release and NF-κB activation with increased expression of cytokines at 72 h. However, only TLR2 upregulation and subsequent NF-κB binding were sustained at 7 days. In vivo, STZ-induced diabetic eNOSKO mice exhibited an increase in tubular TLR2 and HMGB1, but not TLR4, expression [104]. Collectively, the data from in vitro and in vivo experiments so far underscored the pivotal role of TLR2- and MyD88-dependent pathway in prompting and maintaining renal inflammation in DN. Genetic deficiency of TLR2, with other TLRs including TLR4 being intact, significantly abrogates the proinflammatory state and attenuates incipient DN by normalization of microalbuminuria and retention of podocyte number. Both tubular and macrophage TLR2 may perpetuate renal inflammation in STZ-induced diabetic models.

Several factors may contribute to the divergent findings of TLR4 versus TLR2, including a generic difference between human and rodent DN pathologies, the disease duration in
human versus animal models, type 2 DN in human and animals versus type 1 DN in STZ-induced animals, and the use of primary culture versus immortalized cell lines in in vitro studies. Further studies are required to pinpoint the differential roles of TLR2 and TLR4, as well as the relation between MyD88-dependent and -independent pathways in the pathogenesis of type 2 DN and type 1 DN. On the other hand, since TLR2/4 signaling has been implicated in β cell dysfunction and insulin resistance in T1DM and T2DM [105, 106], it could be difficult to differentiate the role of TLR2/4 between the development of diabetes and DN. However, blockade of TLR4 or TLR2 ameliorated renal abnormalities without altering glucose levels [41, 103], which may implicate that TLR4/2 could prompt the progression of DN via renal-specific mechanisms independent of their effects on diabetes per se.

**THERAPEUTIC PERSPECTIVE OF TARGETING TLR SIGNALING IN DN**

The ability of TLR2/4 signaling to initiate and perpetuate the progressive inflammation, albuminuria and fibrosis in DN renders them tempting therapeutic targets for DN. Indeed, a variety of experimental data indicated that current strategies for DN, such as angiotensin receptor blocker [97], aldosterone receptor antagonist [98], statin [107, 108], PPARγ agonist [109], vitamin D analog [110] and dipeptidyl peptidase-4 (DPP-4) inhibitor [111, 112], could exert their pleiotropic effects via inhibiting TLR signaling mediated inflammation. More recently, the sodium/glucose cotransporter 2 (SGLT2) inhibitor empagliflozin, an oral hypoglycemic agent used to treat diabetes by blocking proximal tubular reabsorption of filtered glucose, has been shown to reduce HG-induced TLR4 and NF-κB activation in HK-2 cells [113]. Whether this may translate into renoprotection against diabetic kidney disease remains to be answered by ongoing clinical trials.

In addition to the non-specific approaches to modulating TLR signaling, currently, a variety of novel TLRs antagonists are under vigorous preclinical and clinical trials [114]. The recent report of the crystal structure of TLR4-MD-2 complex has prompted the discovery and development of a number of a lipid A analog, which competes with LPS for binding to TLR4, as a new paradigm for treating sepsis and other inflammatory diseases: (i) Eritoran is a promising TLR4 antagonist that has been shown to be effective in treating inflammation associated with sepsis [115], influenza [116], myocardial I/R injury [117], kidney I/R injury [118] and insulin resistance [119] in experimental animal models. However, Eritoran failed to reduce mortality in the recent phase III ACCESS study for severe sepsis [120]. (ii) CRX-526 is another lipid A mimetic molecule that could exert anti-inflammatory effect in chronic colitis [121] and lung I/R injury [122] models. We have shown that CRX-526 treatment significantly prevented the progression of albuminuria, mitigated parenchymal inflammation and alleviated collagen deposition, implicating the therapeutic potential of CRX-526 in modulating TLR4 signaling in eNOSKO mice with advanced DN [101]. (iii) TAK242 is a TLR4 antagonist that has been discontinued during a phase III trial for severe sepsis other than safety/efficacy reasons [123]. In an experiment on human tubular epithelial cells, TAK242 was shown to inhibit the increase in HMGB1-

**FIGURE 2:** Schema illustrating the role of TLRs in diabetic kidney disease. TLRs in resident renal cells could recognize and respond to the metabolic stress or endogenous ligands activated by the diabetic state, inducing the downstream signaling events to propagate the synthesis proinflammatory cytokines and chemokines, which act as effectors to further facilitate macrophage recruitment and fibroblast proliferation, resulting in a self-perpetuating cycle of renal inflammation, and subsequent tubulointerstitial fibrosis and glomerulosclerosis in diabetic kidney disease. TLRs, Toll-like receptors; HMGB1, high mobility group box-1 protein; S100A8, S100 calcium-binding protein A8.
induced TLR4 signaling and the subsequent NF-κB binding [104]. (iv) Ibudilast (AV411) is a TLR4 antagonist undergoing phase II trial for opioid dependence, which may induce the anti-inflammatory cytokine IL-10 and suppress the inflammatory cytokines TNF-α and IL-6 [124]. Notably, ibudilast was shown to significantly decrease urinary albumin excretion in T2DM patients with microalbuminuria, although the study was not powered to draw a definitive conclusion [125]. (v) GIT27 (VGX-1027) is a TLR2/4 inhibitor undergoing phase I clinical trial. It was shown to confer renoprotective effect in db/db mice via modulating TLR4 signaling [83]. In addition to the synthetic TLR4 antagonists, a number of TLR4 antagonistic antibodies are being developed to block excessive immune response associated with autoimmune diseases, such as colitis and rheumatoid arthritis [114]. On the other hand, two TLR2 antagonists have been identified. OPN-305 is a monoclonal antibody that blocks TLR2 which is now undergoing a phase II clinical trial in preventing delayed graft function, and AP177 was identified as a TLR2 functional aptamer that could significantly inhibit NF-κB activity and suppress the secretion of the cytokines [114, 126]. Moreover, emerging non-traditional approaches for drug discovery such as microRNAs and ubiquitination targeting could be additional potential methods to target against adaptor proteins involved in TLR signaling [114]. On the other hand, tampering the binding between TLRs and their endogenous ligands, such as HMGB1, could be another promising approach [127]. Whether these therapeutic strategies may impact favorably on the progression of DN definitely deserves further studies in animal models of DN.

Of mice and men, due to the considerable differences between the human and murine immune systems, including the cellular expression patterns of TLRs, caution is required when extrapolating findings in murine systems to human disease. Another safety concern is that blocking TLR4, as the classic endotoxin receptor, could have detrimental effects in some infectious and immunological settings. Nevertheless, according to the available results from clinical trials, TLR4 antagonists are well tolerated, and not associated with overt side effects or toxicity. Besides, TLR4 knockout or mutation in mice does not confer lethality or reveal a negative phenotype. Thus, the immune system may have redundant mechanisms in sensing and eliminating bacteria independent of TLR4.

**CONCLUSIONS AND PERSPECTIVE**

In conclusion, the studies described above suggest that TLRs are potent mediators that translate the metabolic derangement of diabetes into functional and structural abnormalities in DN. TLRs in intrinsic renal cells could recognize and respond to the metabolic stress or endogenous ligands activated by the diabetic state, inducing the downstream signaling to secrete proinflammatory cytokines and chemokines, which act as effectors to further facilitate macrophage recruitment and fibroblast proliferation, resulting in a self-perpetuating cycle of renal inflammation, and subsequent tubulointerstitial fibrosis and glomerulosclerosis in diabetic kidney disease (Figure 2). To date, most studies on the role of TLR4 and TLR2 are performed in cultured podocytes and tubular cells. Investigations using mice with kidney cell specific conditional knockout of TLR4 and TLR2 are needed to further pinpoint the in vivo role of TLR2/4 in DN. Moreover, since very few DN models can develop appreciable degrees of tubulointerstitial fibrosis, studies using appropriate DN models with longer observation periods may further strengthen the role of tubular TLR in DN in vivo.

In addition to TLRs on the cell surface, nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) are intracellular sensors for PAMPs and DAMPs which can assemble inflammasome complexes to activate caspase-1 and promote the release of the proinflammatory cytokines IL-1β and IL-18. In diabetic conditions, metabolic syndrome-associated ‘danger signals’ such as islet amyloid polypeptide, high glucose, urate, extracellular adenosine triphosphate (ATP) and fatty acids can be sensed by NLRP3 inflammasome [128]. Although the exact pathway or mechanism of NLR activation in diabetes remains unknown, reactive oxygen species (ROS) seems to be one of the crucial mechanisms of NLRP3 activation because all known NLRP3 activators, including ATP and activators that require phagocytosis, will induce ROS [128]. Recently, the role of several NLR molecules such as NOD2 and NLRP3 in DN has been recognized. NOD2 expression was upregulated in human DN. In vivo, genetic ablation of the NOD2 gene ameliorated renal injury in diabetic mice [129]. In vitro, podocyte NOD2 expression was induced by hyperglycemia, AGEs and TNF-α, and podocyte injury was associated with inflammation and insulin resistance mediated by NOD2 [129]. Additionally, ATP-P2X purinoceptor 4 signaling was shown to mediate HG-induced activation of the NLRP3 inflammasome in HK-2 cell, which could regulate the IL-1 family of cytokine secretion and induce the development of tubulointerstitial inflammation in DN [130]. Inhibition of renal NLRP3 inflammasome activation by quercetin and allopurinol attenuated STZ-induced hyperuricemia/dyslipidemia superimposed nephrotoxicity in rats [131]. Therefore, NLRs may act as intracellular sensors for diabetic stress, and cooperate with TLRs to contribute to the development of DN (Figure 1).

DN is increasingly considered an inflammatory disease in the past decades. Despite promising initial results from certain preclinical treatments that perturb inflammatory and immunological mechanisms, such as PKC inhibitor [132], CCR2/CCR1 antagonism [133–135] and Nrf2 activator [136], which target inflammatory signaling, chemokines or oxidative stress in animal models, their efficacy in human DN remains unknown. Indeed, a number of recent clinical trials failed to prove the efficacy or safety of such therapeutics in human DN [137]. The emerging role of TLRs, together with the recent NLRs and receptor for advanced glycation end products (RAGE), in sensing of diabetic stress and triggering renal injury in DN holds promise for a new paradigm for treating DN.

**ACKNOWLEDGEMENTS**

This work was supported by the National Basic Research Program of China 973 program no. 2012CB517600 (no. 2012CB517606), a General Research Fund from the Research Grants Council of Hong Kong (grant no. HKU 777009M), National Natural Science Foundation of China (grant no. 751...
61200538) and the Hong Kong Cement and Gypsum Co. Ltd. We apologize to those investigators whose work was not cited here due to space limitation.

CONFLICT OF INTEREST STATEMENT

None declared.

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TLRs in diabetic kidney disease


Received for publication: 6.8.2013; Accepted in revised form: 13.9.2013