Review

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Toll-Like Receptors and Diabetic Nephropathy: A Review of Recent Advances

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Abstract

Diabetic nephropathy (DN) is one of the most common kidney diseases, but its exact pathophysiology remains unknown. Toll-like receptors (TLRs) are innate immune receptors that recognize pathogen- and danger-associated molecular patterns, which can result in an inflammatory response. TLR4, TLR2, TLR5, TLR7, TLR8, TLR9, and TLR11 are essential in the pathogenesis of DN, according to recent evidence collected from both *in vivo* and *in vitro* studies. Studies have shown that TLR2 and TLR4 expression is higher in patients with renal failure and nephrotic diabetes. They also play critical roles in podocyte injury and inflammation caused by high glucose. TLR2 and TLR4 may be helpful therapeutic targets to prevent or delay DN in patients with type 2 diabetes mellitus. Additionally, TLR7 may contribute to kidney damage in type 1 diabetes mellitus, whereas downregulation of TLR9 expression inhibits inflammation and apoptosis pathways associated with DN.

Keywords: Toll-like receptor, nephropathy, diabetic kidney disease, chronic kidney disease, TLRs

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Introduction

Diabetic nephropathy (DN) can lead to end-stage renal disease (ESRD). This condition has several consequences, such as loss of glomerular filtration rate, diabetic glomerular lesions, and urine albumin excretion (1-5). DN can develop ESRD in individuals with some stages of diabetes: normal albuminuria, incipient DN, microalbuminuria, and finally, ESRD (6,7). DN risk factors include age, race, genetic profile, hypertension, dyslipidemia, glycaemic control, and tobacco use (8-14).

DN pathophysiology includes hemodynamic and metabolic modifications, oxidative stress, renin-angiotensin system activation, and inflammation; these events overlap and reinforce each other (2). High levels of angiotensin II cause inflammation, advanced glycation end products (AGE), reactive oxygen species (ROS), and dysregulated nitric oxide, which activate signaling pathways such as protein kinase C (PKC), mitogen-activated protein kinase, and nuclear factor kappa light chain enhancer of activated B-cells (NF-κB). Some studies have shown that hyperglycemia via the activation of PKC, especially PKC, inhibits insulin's actions on glomerular epithelial cells. It is also effective against endothelial dysfunction and DN (15-18). Chronic exposure to diabetic conditions can damage kidney cells and induce the release of intracellular damage-associated molecular patterns (DAMPs) into the extracellular space. Pattern recognition receptors (PRRs), such as TLRs, recognize DAMPs (5). Pathogen-associated molecular patterns (PAMPs) are various microbial molecules that share recognizable biochemical attributes (entire molecules or, more often, part of molecules or polymeric assemblages) that alert the organism about the existence of threatening pathogens. Such exogenous PAMPs are recognized by cells of the innate and acquired immunity system primarily through toll-like receptors (TLRs), which activate several signaling pathways, among which NF-κB is the most specific.

Consequently, some cells are activated to release pathogens and pathogen-infected cells. An immunological response is triggered to generate and select specific T-cell receptors and antibodies that are best suited for later identification of pathogens. Most of the responses triggered by PAMPs fall into the general categories of inflammation and immunity. Therefore, PAMPs constitute a more prominent family of damage-associated molecular patterns, or DAMPs (19).

TLR1, TLR2, TLR4, TLR5, and TLR6 are receptors for microbial particles on the cell surface, and TLR3, TLR7/TLR8, and TLR9 are receptors for nucleic acids in intracellular endosomes (19-21). Immune cells, lungs, heart, liver, intestines, and kidney cells express TLRs and can play roles in various pathologies (22-25). TLR1, TLR2, TLR3, and TLR4 are present in kidney, mesangial, and tubular epithelial cells, whereas podocyte and Bowman's capsules express TLR4 (26-30).

TLR2 and TLR4 can recognize endogenous ligands; some of these endogenous ligands released by kidney cells in patients with diabetes are suggested to be responsible for DN via TLR2/4-mediated NF-κB activation (24,29,31-43).

TLRs interact with several cytosolic adaptor molecules, including myeloid differentiation primary-response protein 88, and have an intracellular Toll-interleukin (IL) 1 receptor domain (MyD88). TLR1, TLR2, TLR5, and TLR6 activate MyD88, which only induces NF-κB. TLR7, TLR8, and TLR9 activate NF-κB and interferon regulatory factor-7 (IRF-7) (44), generating interferon alpha. TLR3 interacts with Toll-IL 1 receptor domain-containing adaptor protein-inducing interferon (IFN)-β (TRIF) by activating only interferon regulatory factor-3 (IRF-3) (45), activating both IFN-β and NF-κB. TLR4 interacts with both TRIF and MyD88, and TRIF induces IFN-β and NF-κB, whereas MyD88 only induces NF-κB (44,46-49). Thus, TLRs and other PRRs promote the synthesis and release of interleukins, cytokines, chemokine, and adhesion molecules; these pro-inflammatory agents lead to inflammation.

Monocyte chemoattractant tumor necrosis factor-alpha (TNF-α), protein-1 (MCP-1), IL-1, IL-6, IL-18, and intercellular adhesion molecule-1 are involved in DN pathogenesis (49).

TLR2 and TLR4 and their downstream molecules are targeted as therapeutic targets because of their roles in DN (50). Some TLR antagonists are in clinical trial phases or are being investigated in animal models, and some of them have shown promising effects, but some have still had no significant results.

We conducted this review to elucidate the roles of TLRs in DN pathophysiology and identify recent advancements in this topic. Recognizing novel therapeutic strategies for inflammation can help prevent or delay DN.

Methods

This article reviewed the literature on the relationship between TLRs and DN in three databases, including PubMed, Scopus, and Google Scholar, up to July 2024. Search keywords included diabetic, TLR, TLR4, TLR2, TLR5, TLR7, TLR8, TLR9, TLR11, chronic kidney disease, ESRD, nephropathy, and kidney. End note 20 was used to eliminate duplicates. Keywords were searched in English-language journal articles until July 2024, and the reference lists of the included articles were manually searched.

Literature Review

TLR Signaling Pathway

Based on location, TLRs are divided into extracellular and intracellular groups. The extracellular TLRs TLR1, TLR4, TLR5, TLR6, and TLR11 are located on the cell membrane's outer surface. In contrast, intracellular TLRs include TLR3, TLR7, TLR8, and TLR9, which recognize endosomal ligands (51). The TLR signaling pathway mainly includes the MyD88 and non-MyD88 pathways. We provide a summary of these routes as follows:

MyD88 Pathway

TLRs (except TLR3) initiate their signaling pathway by binding to specific ligands and activating the MyD88 protein. Once bound to their ligands, extracellular TLRs bind to the TIRAP domain of MyD88 proteins through their intracellular terminal called toll/IL1 receptor (TIR). The other domain of MyD88 (52), after interaction with IRAK4 and TRAF6, triggers a cascade reaction that ultimately produces NF-κB (53). By entering the cell nucleus, NF-κB increases the expression of inflammatory cytokines, including IL-1, IL-6, and TNF-α. Similarly, in the signaling pathway of intracellular TLRs, activation of MyD88, IRAK4, and TRAF3 ultimately leads to an increased expression of IFN-1 (54).

TRIF Pathway

TLR3 and a particular type of TLR4, which endocytose after binding to their ligands, can utilize a signaling pathway independent of MyD88 (48). In this pathway, the cytosolic terminals of TRF 3 and 4 are connected to the TRAM domain of the TRIF protein. This cascade reaction increases the IFN-β production by activating TRAF3, IKKi, and TBK1 (44). Additionally, the expression of inflammatory cytokines increases with the activation of RIP1 and TRAF6 (54).

TLR2

Experimental Studies

It has recently been reported that TLRs are activated by non-microbial endogenous ligands (55). Therefore, increased TLR levels in kidney cells and leukocytes are considered the leading causes of acute and chronic kidney diseases (56).

Devaraj et al. (33) used streptozotocin (STZ) to induce type 1 diabetes mellitus (T1DM) in TLR2-knockout mice (TLR2-/-) and wild-type littermates (C57BL/6J-WT) (STZ). In diabetic mice (WT+STZ) compared with non-diabetic WT mice, macrophage TLR2 expression and MyD88-dependent signaling were increased. In diabetic TLR2/macrophages, these indicators were diminished (33). Compared with WT mice, WT+STZ mice had an upregulated kidney body weight ratio due to cell hypertrophy, podocin, decreased kidney nephrin, increased podocyte number, increased TGF-β, albuminuria, and laminin. In comparison with WT+STZ mice, the kidneys of TLR2/+STZ mice had lower amounts of TGF-β and laminin (57). In WT+STZ mice, most macrophages in the kidney and peritoneum had the M1 phenotype; this was diminished in TLR2/+STZ mice. TLRs activate MyD88-dependent and MyD88-independent downstream signaling pathways, respectively. TLR2 signaling primarily causes inflammation via the MyD88-dependent pathway. They demonstrated that deletion of TLR2 significantly reduces inflammation caused by diabetes because TLR4 levels and its non-MyD88-dependent signaling proteins (IRF-3) were unaffected (30). MyD88, NF-B activity, and IRAK-1 phosphorylation were significantly decreased in correlation with the release of pro-inflammatory cytokines and chemokine (52). Abrogation of inflammation in diabetes using TLR2 as a target suggests an acceptable therapeutic strategy for reducing inflammation, which is also associated with DN. Despite other TLRs, genetic TLR2 deficiency significantly retarded the proinflammatory state of T1DM for up to 14 weeks and reduced early DN at the cellular level (33).

Li et al. (37) investigated increased TLR2 expression in rat kidneys in patients with DN. Furthermore, they showed that after a short duration of 2 weeks, inflammation biomarkers in TLR2KO STZ mice were reduced, and wound healing parameters were enhanced. Statins, PPAR-gamma agonists, and angiotensin receptor blockers decrease the expression of TLR4 and TLR2, respectively (57).

Zhang et al. (58) showed that TLR2 expression was increased noticeably in diabetic rats' kidneys, whereas treatment with total glucosides of peony (TGP) (extracted from *Paeonia lactiflora*, a Chinese traditional herbal medicine) decreased it. Their study showed that the TGP could prevent renal tubulointerstitial injury in diabetic rats. This study found that renal tubular epithelial cells in STZ-induced diabetic kidneys overexpressed TLR2, which is associated with ED-1-positive macrophage activation. Additionally, the findings supported tubular epithelial-myofibroblast trans differentiation and tubulointerstitial inflammation. Furthermore, treatment with TGP reduced macrophage infiltration in diabetic rats (58).

Data presented by other researchers also proved that TLR2 promotes tubulointerstitial inflammation and macrophage activity and aggravates tubular epithelial-myofibroblast trans-differentiation and renal fibrosis (39). In slightly earlier STZ-induced diabetic injuries, spironolactone ameliorated renal fibrosis, potentially by inhibiting macrophage infiltration and TGF-1 production (59). The TGP reduced the exhibition of TNF-α and

IL-1 in DN rats' kidneys (60). TNF-α and IL-1 are pro-inflammatory cytokines that are mainly produced by activated macrophages and leukomonocytes (52).

Wu et al. (61) attempted to clarify the effect of the suppressor of cytokine signaling 1 (SOCS1) on DN by regulating the TLR signaling pathway in the Sprague-Dawley rats. STZ-induced DN leads to the necrosis of pancreatic β-cells in rats, resulting in the degeneration and loss of function of of secreting insulin (62). STZ significantly increases the fasting blood glucose level and has been applied to establish a DN animal model (63). Increased levels of creatinine, blood glucose, blood urea nitrogen (BUN), and uric acid are markers of DN. The findings suggested that TNF, IL-6, and IL-1 levels were elevated in the DN group, indicating an increased inflammatory response. The overexpression of SOCS1 alleviated the above pathological conditions, suggesting that SOCS1 has an excellent protective effect against DN. SOCS1 was abnormally downregulated in the kidney of DN mice (64). A study by Mudaliar et al. (41) also showed that during the development of renal disease, the expression of essential indicators such as interferon-gamma (IFN-γ) and TLR2 and MyD88 pathway genes was significantly decreased in the SOCS1 overexpression group, indicating that SOCS1 overexpression can improve renal injury (41). Therefore, SOCS1, a critical regulator of the TLR signaling pathway, appears to be an effective target for treating DN injury.

Focusing on the role of CLOCK genes in nephropathy, Xu et al. (65) investigated the effect of melatonin on this process in diabetic mice. They discovered that among the CLOCK genes, DEC1 had the most significant effect on activating the TLR2/MyD88/NF-κB signaling pathway. By regulating these genes, melatonin can reduce the TLR2 signaling pathway and slow down the progression of DN (Figure 1) (65).

TLR4

Experimental Studies

Duan et al. (66) reported that extracellular vesicles made from adipose-derived mesenchymal stem cells (ADSCs) contain miR-26a-5p, which blocks DN in mice. They recognized different expression levels of miRNAs in extracellular vesicles from these cells in DN and predicted downstream regulatory genes using bioinformatics analyses. They found that high glucose (HG), ADSC-derived extracellular vesicles, the miR-26a-5p inhibitor, TLR4 plasmids, and the NF-κB pathway activator were toxic to MP5 and mouse glomerular podocyte cells (phorbol-12-myristate-13-acetate). They concluded that although TLR4 production was high, miR-26a-5p expression was deficient. Significantly, extracellular vesicles decreased the pathological symptoms of DN in diabetic mice and transfected miR-26a-5p into hyperglycemic MP5 cells, promoting cell survival while preventing apoptosis in MP5 cells. They also observed that miR-26a-5p inhibited the NF-B pathway, downregulated vascular endothelial growth factor A, and targeted TLR4 to protect MP5 cells from excessive hyperglycemia (VEGFA). They also found that in HG-induced MP5 cells, miR-26a-5p transported by extracellular vesicles produced from ADSCs targeted TLR4 and inhibited the NF-κB/VEGFA pathway. TLR4 was elevated in diabetic rats, making them more susceptible to acute kidney damage caused by myocardial infarction. These studies support their findings that TLR4 promotes the pathogenesis of DN (66).

Ding et al. (67) discovered that hydrogen sulfide (H2S) plays a protective role against DN. They investigated small interfering RNA to destroy TLR4 expression, whereas the specific inhibitor LY294002 suppressed PI3K function-glucose-induced significant mesangial cell proliferation by inhibiting endogenous H2S synthesis in a TLR4-dependent manner. Exogenous H2S treatment with NaHS significantly reduced the hyperproliferation of MMC. In addition, inhibition of the PI3K/Akt pathway considerably improved cell proliferation (67). TLR4 knockdown did not significantly alter the inhibition of HG-induced cystathionine-γ-lyase expression (67). However, TLR4 silencing significantly abrogates cell over proliferation following cystathionine-γ-lyase depletion. These results indicate that TLR4 is downstream of the cystathionine-γ-lyase pathway in mesangial cells induced by hyperglycemia during proliferation (67).

Zhang et al. (68) showed that in hexokinase 2 (HK-2) cells treated with HG levels, miR-124 released blocked the activation of the TLR4/NF-κB signaling pathway. In HK-2 cells exposed to HG levels, the inactivation of the NF-κB signaling pathway by the inhibitor pyrrolidine dithiocarbamate (PDTC) reduced the expression of type IV collagen (Col4) and fibronectin. On the other hand, DN is determined by mesangial matrix expansion followed by glomerulosclerosis, leading to the aggregation of extracellular matrix (ECM) components secreted mainly from mesangial cells and Col4, which is one of the significant components of increased ECM in DN (69,70). In HK-2 cells treated with HG, baicalin suppressed the activation of the TLR4/NF-κB pathway and increased miR-124, which prevented renal fibrosis (68).

In 2015, Jheng et al. (71) experimented on albumin stimulating renal tubular inflammation through the heat shock protein 70 (HSP70)-TLR4 axis in mice with early DN. One aspect of DN is increased urinary albumin excretion. The scientists concluded that endogenous ligands released in response to albuminuria cause inflammation of tubular cells through TLR signaling, which worsens the progression

and severity of renal injury in DN. They found that the absence of TLR4 (but not TLR2) reduced diabetes-related changes, such as albuminuria, renal inflammation, tubular interstitial fibrosis, and tubular apoptosis. In TLR4/diabetic mice, protection from decreased tubular injury is related to renal injury but not to improving glomerular lesions. While looking for potential endogenous TLR ligands, the authors discovered that diabetic mouse-injured tubules had significantly higher HSP70 levels. The expression of inflammatory mediators induced by albumin is reduced by HSP70 inhibition. Additionally, in a TLR4-dependent manner, HSP70 stimulates the synthesis of inflammatory mediators. The authors discovered that all participants with DN had HSP70 and TLR4 elevated in injured tubules. Inhibiting tubular inflammation with medications targeting the albumin-HSP70-TLR4 axis may be a novel therapeutic approach to stopping the progression of DN (71).

Ishibashi et al. (72) experimented with semaphorin 3G, a new podocyte gene that protects glomerular podocyte against lipopolysaccharide-induced inflammation. When mice were administered lipopolysaccharide to generate acute inflammation or STZ to cause hyperglycemia, semaphorin 3G deficiency increased albuminuria and the production of inflammatory cytokines, such as chemokine ligand 2 and IL-6. In addition, semaphorin 3G expression was reduced by inhibiting TLR4 signaling induced by lipopolysaccharide. Therefore, podocytes release semaphorin 3G protein to protect themselves against inflammatory kidney diseases (72). Semaphorin signals are proposed to participate in innate and adaptive immune reactions. For example, semaphorin 3A-plexin A4 enhances TLR4 signaling (73). Semaphorin 3A is the best-characterized hemaphorin among the hemaphorins in DN, and urinary hemaphorin 3A is associated with diabetic proteinuria and, as a mediator of DN, causes associated inflammation in mice (74). Thus, increased semaphorin 3A expression may worsen DN through increased expression of inflammatory cytokines. Furthermore, it has been observed that semaphoring 7A and its receptor plexin C1, in addition to semaphorin 3E (75), promote inflammation (76).

Cha et al. (77) investigated the renal protective effects of TLR4 blockade in mice with type 2 diabetes mellitus (T2DM). For 12 weeks, they administered (S, R)-3-phenyl-4,5-dihydro-5-isoxazole acetic acid (GIT27) to 8-week-old db/db mice. The TLR4 and TLR2/6 signaling pathways mediated by macrophages, were inhibited. GIT27 therapy reduced insulin resistance and improved blood glucose regulation. In the db/db control group, GIT27 therapy significantly reduced the excretion of urine albumin, the production of pro-inflammatory cytokines, the metabolism of tissue lipids, the level of oxidative stress, and the development of glomerulosclerosis. The findings showed that administration of GIT27 improved insulin sensitivity and provided renal damage protection in type 2 DN via the metabolic and anti-glomerulosclerosis pathways. Additionally, it considerably reduced lipid

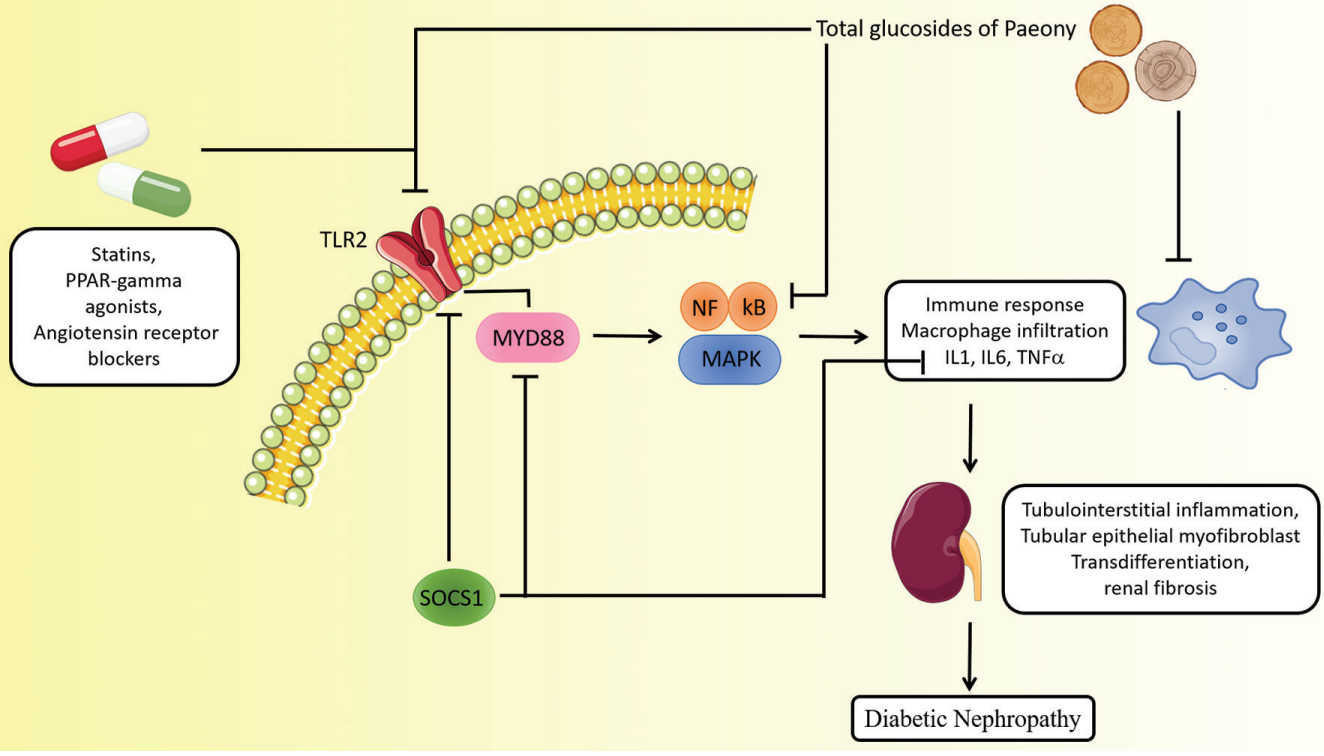

Figure 1. Stablished a summary of cellular mechanisms mediated by TLR2.
TLR2: Toll-like receptor 2, IL: Interleukin, TNF-α: Tumor necrosis factor alpha, NF: Nuclear factor, kB: Kappa B, MAPK: Mitogen-activated protein kineases, PPAR: Peroxisome proliferator-activated receptor

abnormalities, significantly improved hepatic steatosis, and caused adipose morphologies to transform into less distinct forms. These findings suggest that blocking the TLR signaling pathway may protect individuals with diabetes (77).

Recent studies suggest that TLR4 and T helper 17 (Th17) cells may have essential functions in immunopathogenic processes as follows: 1- $CD4^+$ T-cell activation and IL-17 upregulation are mediated by TLR4 overexpression caused by microbial pathogens, inflammatory cytokines, and endogenous molecules; 2- An inflammatory response induced by upregulation of $CD4^+$ T-cells cytokines, causing an increase in the synthesis of endogenous compounds and inflammatory cytokines; 3- Initiation of the downward cycle of exacerbation of chronic infection in renal tissue in DN, due to upregulation of TLR4, induced by overproduction of inflammatory cytokines and $CD4^+$ T-cell (62).

The activity of TLR4 is responsible for lipopolysaccharide, high-mobility group box1, hyperglycemia, oxidative stress, and AGE. TLR4 stimulation activates kappa B, which is the primary regulatory transcription factor that controls IL-6 (78). According to a study by Zabad et al. (79), p-coumaric acid (P-CA) is a phenolic acid (member of the hydroxycinnamic acid family) with significant anti-inflammatory activity. Additionally, P-CA significantly reduced the levels of renal malondialdehyde, IL-6, TLR4, TGFβ1, and collagen compared with the DN group. These results provide P-CA protection against DN progression (79).

Yang et al. (80) studied the effect of SOCS2 in DN rats by inhibiting the TLR4/NF-κB pathway. They analyzed the NF-κB, TLR4, and SOCS2 levels in the renal tissues of control and DN rats. Western blotting showed that the SOCS2 abundance was significantly low and that TLR4 was dramatically high in the renal tissues of DN rats. The overexpression of SOCS2 reduced DN development by affecting the TLR4/NF-κB pathway, which helped generate new therapies for DN (80).

Feng et al. (81) examined the impact of acetonate on the expression of IL-8 and TLR4 in the renal tissues of DN rats. Artesunate reduces 24-h proteinuria and inhibits inflammation in DN mice by upregulating IL-8 and TLR4 expression. It also alleviated pathological nephron lesions in rats with DN (81).

Han et al. (82) suggested that the Hongoki capsule reduced renal tubular epithelial-mesenchymal transition in DN by inhibiting NOD-like receptor (NLR) family pyrin domain containing 3 (NLRP3) inflammasome activation and TLR4/NF-κB signaling. Huangkui capsules are modern Chinese anti-inflammatory drugs that are widely used in the clinical treatment of DN. Epithelial-tube mesenchymal transition to the kidney is a significant pathogenesis of renal interstitial fibrosis in DN. The physiological roles of NLRP3 inflammasome activation and TLR4/NF-κB signaling are linked to epithelial-to-mesenchymal transition. Their study was conducted to elucidate the therapeutic effects of Hongoki capsules on the epithelial-mesenchymal transition of renal tubules in DN and its underlying mechanism *in vivo* compared with rapamycin. The results of their study further indicate that the Hongoki capsule may reduce the renal tubule epithelial-to-mesenchymal transition in a rat model of DN, possibly due to the NLRP3 infrastructure in the kidney. This effect was made possible by inhibiting TLR4/NF-B signaling (82).

Yu et al. (83) investigated the inhibitory effects of rapamycin on TLR4 and IL-17 in the early stage of rat DN. They divided 18 diabetic rats into three experimental groups randomly: Six in the DN group, six in the rapamycin (rapa)-a treated group for DN, and six in the control group. Their study showed higher TLR4 protein expression in the DN group than in the control group. The expression of TLR4 in the rapa group decreased after rapamycin administration. In addition, these new findings confirmed the critical function of TLR4 in renal fibrosis (one of the primary causes of DN progression) (83).

Gong et al. (84) investigated the impact of diabetes on renal ischemia and reperfusion injury in rats. In this study, STZ-induced diabetic and control rats were examined. Oxidative stress was determined by western blotting. Compared with control animals, heme oxygenase-1 (HO-1), nuclear factor-erythroid two factors 2 (NRF2), TLR4, and NF-κB were among the proteins connected to inflammation. Ischemia/reperfusion (I/R)-exposed diabetic rats displayed more severe renal failure and tube damage. Diabetes increases oxidative stress, inflammation, and apoptosis after renal I/R by increasing TLR4/NF-κB signaling and decreasing the Nrf2/HO-1 pathway. Nuclear TLR4 and NF-κB levels were greatly enhanced by I/R damage in the diabetic group but were not significantly elevated by hyperglycemia alone, compared to the regular sham (NS) group ($p=0.05$) (84).

Endogenous ligands released by stressed or injured tissues, such as DAMPs and high mobility group box 1 (HMGB1), activate TLR4 signaling (71). In renal tubules, I/R injury triggers the generation of DAMPs and initiates signaling pathways that amplify the initial harm caused by NF-κB-mediated inflammation (85). TLR4/NF-κB signaling is initiated in the early stages of DN through the overexpression of HMGB1, which causes cytokine production, inflammation, and proteinuria (86).

In diabetic rats, more nuclear TLR4 and NF-κB levels were found (86). The TLR4-NF-κB axis was more active in diabetic rats following I/R compared to the healthy control group (86). These findings imply that elevated

TLR4/NF-κB-mediated inflammatory signaling is linked to more significant renal I/R damage (RI/RI) susceptibility in diabetic rats (87).

Ashrafi Jigheh et al. (88) found that empagliflozin reduces oxidative stress and kidney inflammation in STZ-induced diabetic rats partially by suppressing the HMGB1-TLR4 receptor axis. As an inhibitor of sodium-glucose cotransporter II (SGLT2), empagliflozin has anti-oxidative and anti-inflammatory effects on DN. This study aimed to assess the impact of empagliflozin on the renal levels of HMGB1 and its similar receptor TLR4 in STZ-induced diabetic rats. These studies showed that the essential inflammatory cytokine HMGB1, which is secreted by activated and necrotic cells and its associated receptors TLR4 and RAGE, can be reduced by empagliflozin. Additionally, an increase in kidney function was observed with a decrease in serum levels of urea and creatinine (88).

In another study, Awad et al. (89) measured the effect of 8 weeks of BS-independent Montelukast treatment on DN in STZ-induced (55 mg/kg) diabetic rats. In this study, in addition to improving renal factors (e.g., BUN and Cr), the expression of TLR4 and NF-κB also decreased (89). Similarly, Shelke et al. (90) demonstrated that treatment with phloretin and losartan improved BUN and Cr in diabetic rats treated with AKI. This combination therapy preserved renal function by inhibiting inflammatory processes related to TLR4 (90).

In addition, studies have shown that the effects of astragalus polysaccharide (91), bergenin (52), sulbutiamine (92), Danzhi Jiangtang capsule (93), and sanziguben (94) on improving kidney factors and inhibiting the TLR4 signaling pathway are similar. Moreover, overexpression of quinone oxidoreductase 1, which is reduced in diabetes, reduces the activation of the TLR4/NF-κB signaling pathway and TGF-β1 (95).

Inhibition of the TLR4/MyD88/NF-κB signaling pathway by DACH1 reduces renal inflammation and fibrosis induced by palmitic acid in DN, as shown in Lin et al. (96).

In their study, Yu et al. (97) revealed the effect of the TLR4/MyD88/NF-κB signaling pathway in DN. In this study, the expression of HMGN1, a DAMP protein, and TLR4 was increased in STZ-induced diabetic mice. HMGN1 downregulation decreased TLR4 and renal interstitial macrophages. In contrast, HMGN1 administration had a negative effect. However, TLR4 inhibition reduced its adverse effects (97).

Another recent study reported interesting results by examining mitochondrial function as a factor in nephropathy (98). Thus, the overexpression of RUNX3 improved the function of mitochondria and reduced the apoptosis rate. In contrast, TLR4 overexpression prevented these benefits by activating NF-κB (98). Similarly, Wu et al. (99) displayed that increased peroxiredoxin 6 levels can enhance mitochondrial function and inhibit the activation of the TLR4/NF-κB pathway in diabetic models. In addition, peroxiredoxin 6 reduces oxidative stress and apoptosis in kidney cells (99).

Clinical Studies

Yang et al. (100) collected the anti-inflammatory results of 1,25-dihydroxy vitamin D3 in monocytes extracted from the serum of patients with DN and uremia, considering TLR4 and NFκB p65. These findings indicated that the TLR4/NF-κB p65 signaling pathway was connected to the anti-inflammatory effects of vitamin D3 in inflammatory immune responses in patients with DN and T2DM (100).

Verzola et al. (101) examined the *TLR4* gene and expression of the protein, TLR4 downstream signaling molecules (p-p65 and phospho-IκBα), and TLR4 target genes (TNF-α, tumor necrosis factor receptor 1 (TNFR1), IL-6, C-C chemokine receptor type 2 (CCR2), chemokine (C-C motif) ligand 2 (CCL2), C-C chemokine receptor type 5 (CCR5), chemokine (C-C motif) ligand 5 (CCL5), CD4, CD8, CD45, and CD68 in kidney biopsies from 4 groups, including 12 patients with T2DM and microalbuminuria, 13 patients with minor renal mass surgery, 11 with overt DN, 10 with minimal change disease (MCD), and the control kidneys, to determine whether immune activation via TLR4 signaling in resident renal cells can convert the microalbuminuric form of DN to overt DN. TLR4 (mRNA and protein) was upregulated in glomeruli and tubulointerstitium in both microalbuminuria and overt DN compared to the MCD group and control kidney. The percentage of p-p65-positive cells was increased in patients with microalbuminuria (mainly in glomerular nuclei and podocyte nuclei) and with overt DN (mainly in glomerular nuclei and tubular nuclei) compared with minimal change disease (MCD) and control kidneys. In addition, the expression of phospho-IκBα (protein) and TNF-α (mRNA and protein) was increased in the glomeruli and tubules in microalbuminuria. Overt DN and TNFR1 expression (mRNA) was increased in the tubules of microalbuminuria and in both tubules and glomeruli of overt DN compared with minimal change disease (MCD) and control kidneys (101). Gene expressions of TNFR1, TLR4, TNF-α (in the tubulointerstitial compartment), and IL-6 (in the glomeruli) are linked to the rate of subsequent renal function loss (101).

Wang et al. (102) found that tubular damage was associated with the upregulation of TLR4 and gasdermin D (GSDMD) in patients who have diabetic kidney disease. Furthermore, they found that TLR4 inhibition protected tubular cell damage by suppressing the pyroptosis associated with GSDMD in db/db mice. Their study also showed that

TLR4/NF-κB and GSDMD-NT expression in HK-2 cells under an HG environment was increased, and inhibition of TLR4/NF-κB signaling, in addition to reducing proptosis, also reduced the expression of GSDMD-NT and caspase-1 in HK-2 cells under HG medium. Their results showed that HG levels increased TLR4 expression, caspase-1 cleavage, GSDMD, and IL-1β secretion, which might be fractionally reversed by NF-κB and TLR4 inhibitors. Additionally, NF-κB activation can be reduced by TLR4 inhibition. Their results showed that tubular cell proptosis in diabetic kidney disease, the diabetic rat model, and HK-2 cells are modulated by TLR4/NF-κB signaling. Experiments have shown that GSDMD-associated proptosis activation is connected to TLR4/NF-κB signaling in tubular cells and that TLR4/NF-κB signaling suppression reverses enhanced GSDMD-NT expression in high-glucose environments (Figure 2) (102).

TLR2 and TLR4

Experimental Studies

Zhang et al. (103) explored the renoprotective effects of paeoniflorin. Paeoniflorin was administered intraperitoneally to db/db mice. Extracellular vesicles were immunostained for TLR2, TLR4, CD68, NF-κB p65, mRNA expression of inflammatory factors, and TLR2/4 signaling proteins. Their findings demonstrated that compared with the db/db group *in vivo*, paeoniflorin decreased the urine albumin excretion rate and limited the infiltration and activation of macrophages by blocking the TLR2/4 pathway (66).

Kaur et al. (104) examined the expression of TLR2, TLR4, and the activation of its downstream signaling molecules in mouse mesangial cells (MMC) under hyperglycemic conditions to test their hypothesis that the mesangium's TLRs may be crucial players in nephropathy and mesangium expansion. They observed the mRNA expression of TLR4, MyD88, TRAM, and IRF3, mean fluorescence intensity (MFI) of TLR4 surface expression, and NF-κB p65 activity to be significantly increased in the presence of hyperglycemia compared with normal glucose levels and the mannitol control (104). However, similar to previous studies, they observed no increased expression of TLR2 (39,104).

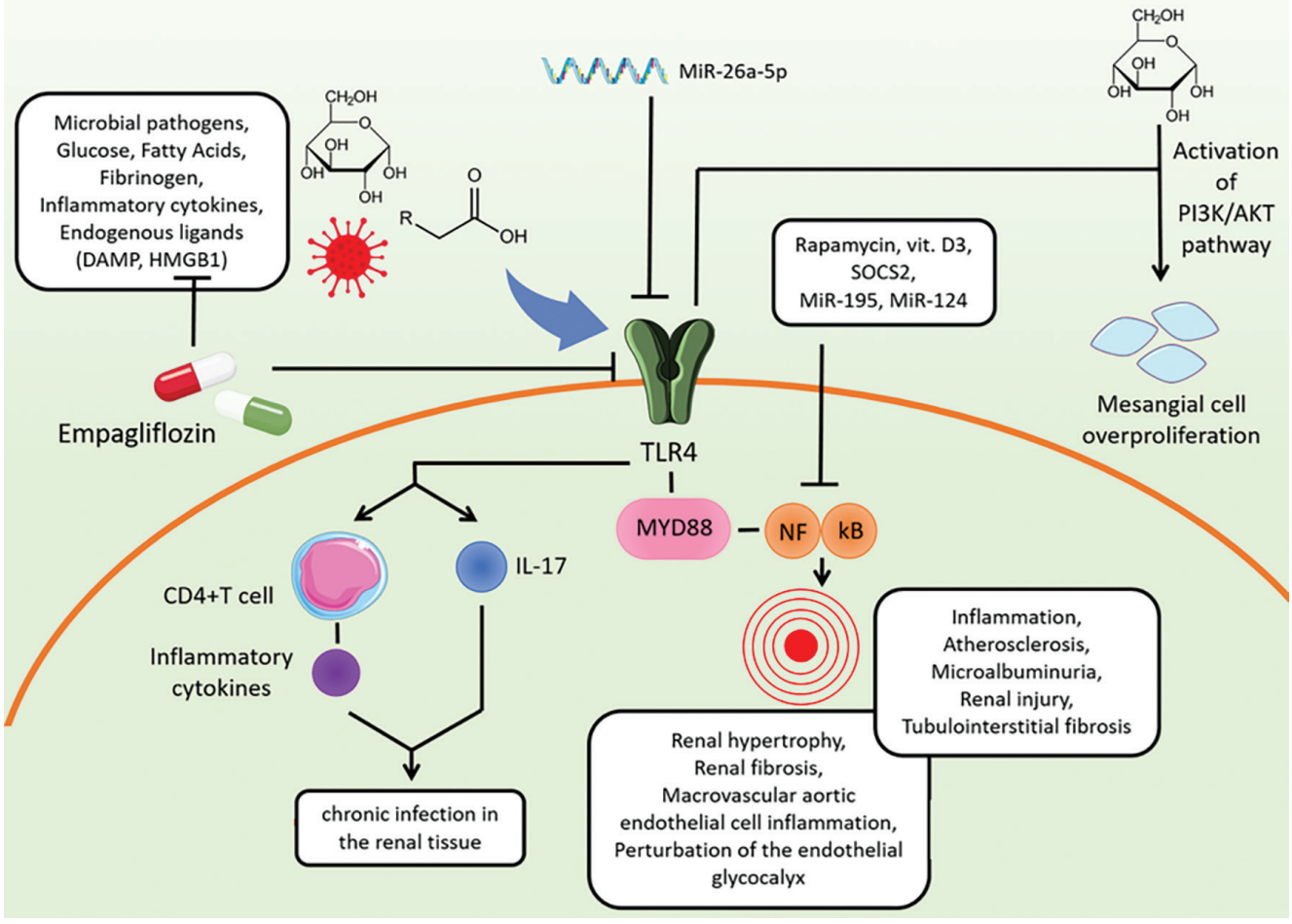

Figure 2. Stablished a summary of cellular mechanisms mediated by TLR4.
DAMP: Damage-associated molecular pattern, HMGB1: High mobility group box-1, IL: Interleukin, NF: Nuclear factor, kB: Kappa B, TLR4: Toll-like receptor 4, PI3K/AKT: Phosphatidylinositol-3-kinase

Devaraj et al. (105) reported that hyperglycemia caused a rise in ROS, possibly caused by the activation of oxidases, which induced NF-κB activity. In addition, obesity-activated NF-κB has been associated with inflammation and oxidative stress. Some studies have shown that patients with T1DM and T2DM have higher levels of TLR2 and TLR4 expression and activity in their monocytes, along with related inflammatory markers (22,105,106). TLR4 increased NADPH oxidase activity and ROS production (22,105-107). More studies have shown that plaques from TLR4 knockout (TLR4 KO) mice have smaller lesions, less lipid content, and more macrophage infiltration (108). Moreover, TLR4 KO diabetic mice showed significantly reduced MyD88, IRAK-1 protein phosphorylation, IRF3, TRIF, and NF-κB activity, and the release of inflammatory cytokines (IL-1, IL-6, IL-8, interferon gamma-induced protein 10 (IP-10), IFN-γ, MCP-1, and TNF-α) in comparison to wild-type diabetic mice (108).

In another study, Zhou et al. (109) demonstrated that TGP suppresses dendritic cell (DC) maturation by blocking TLR4 and five pathways, thereby lowering the proliferation of antigen-driven T-cells and the generation of pro-inflammatory cytokines (IL-1, TNF-α, IL-6, and IL-12) (109).

Kajiwara et al. (110) examined the effect of TLR4 blockade on the development of DN caused by *Porphyromonas gingivalis* lipopolysaccharide (Pg-LPS). Findings showed higher urea nitrogen and creatinine levels in diabetic mice receiving Pg-LPS than in those without Pg-LPS, while erythroid (a TLR4 blocker) slightly reduced urea nitrogen and creatinine levels. TLR2 expression was present in diabetic rats, with higher levels in the administered Pg-LPS group. TGF-b was found to be more abundant in diabetic mice administered Pg-LPS. Compared with diabetic mice that were not administered Pg-LPS, type 1 collagen levels were higher in Pg-LPS-treated diabetic mice. When fed erythroid and Pg-LPS, this expression was less intense in diabetic rats. TLR2-positive glomeruli in diabetic mice and diabetic mice receiving Pg-LPS contained signal transducer and activator of transcription 3 (STAT3), but not in TLR2-negative glomeruli in non-diabetic mice that received Pg-LPS. When diabetic rats were administered Pg-LPS, more glomeruli exhibited TLR2 and STAT3 expression than those without Pg-LPS. TLR2 and TLR4 have been implicated in the promotion of nephropathy caused by *P. gingivalis* (110). The membrane component of LPS enters the peripheral circulation, links the hepatic intestinal circulation to the systemic circulation, and activates circulating leukocytes via TLR2 and TLR4. Hence, they secrete inflammatory cytokines (52,111-114) and promote the progression of nephropathy. The TLR4 blocker erythroid may partially activate glomerular endothelial cells by recognizing intestinal bacterial LPS through TLRs (110).

Clinical Studies

The study by De Melo et al. (115) aimed to investigate the development of DN and the TLR inflammatory cascade in children and teenagers with T1DM. Their study showed that the mRNA expression of MyD88, NF-κB, TLR2, and TLR4 was noticeably increased in the T1DM group compared with the normoglycemia group. This suggests that these genes are early mediators of the onset of diabetic kidney disease in children with T1DM (115).

In the study by Wang et al. (116), human umbilical cord mesenchymal stem cells (HUC-MSCs) were co-cultured with podocyte and implanted into DN mice. In their study, they have found that HG decreased podocyte viability, activated TLR2, and four signaling pathways, and increased the expression of inflammatory cytokines in podocyte and DN mice. On the other hand, HUC-MSCs can lessen inflammation and restrict the TLR signaling pathway induced by HG. HG increased TLR2 and TLR4 expression at both the mRNA and protein levels (116).

Zhang et al. (117) investigated the protective effect of total peony glucosides on DN, which was linked to the inhibition of TLR2 and TLR4 activation (117). Their research revealed that paeoniflorin therapy reduced proteinuria and increased the rate at which db/db mice cleared creatinine (103). The paeoniflorin group's inhibition of inflammatory cytokines and chemokine (TNF-α, MCP-1, IL-1, and iNOS) combined with blocking NF-κB activation and macrophage recruitment was remarkably compatible with the findings of Fu et al. (118). They found that iNOS, a crucial marker of M1 macrophages (which, in contrast to M2 macrophages, increase the inflammatory response and tissue injury), could predict the degree of inflammation in activated macrophage populations in DN models (103). Their studies confirmed Devaraj et al.'s (32) findings that AGE exposure enhances iNOS expression in db/db animals and the M1 phenotype in RAW264.7 cells (32). They demonstrated that paeoniflorin therapy decreased macrophage recruitment and the M1 phenotype in a dose-dependent manner, which decreased the production of proinflammatory cytokines, consistent with their *in vitro* experiments. They therefore have grounds to believe that paeoniflorin's protective effect on DN is likely connected to its ability to inhibit the activation of the M1 macrophage phenotype. In their subsequent experiment (119), they treated AGE-induced RAW264.7 macrophages with oxidation of 1-palmitoyl-2-arachidonoyl-sn-glycerol-3-phosphorylcholine (OxPAPC)- a TLR signaling inhibitor that is only effective against TLR2/4 (119). TLR2, TLR4, MyD88, phospho-IRAK1 (p-IRAK1), phospho-interferon regulatory factor 3 (p-IRF3), and NF-κB p65, as well as

TNF-α, IL-1, MCP-1, and iNOS were statistically reduced, indicating TLR-mediated macrophage activation and TLR-inhibited paeoniflorin treatment (103).

TGP have anti-inflammatory and immunomodulatory effects, as reported by Zhang et al. (117). However, the connection between TLRs and TGP in DN remains unclear. TGP significantly inhibited the expression of TLR2 and TLR4, p-IRAK1, MyD88, p-IRF3, nuclear factor NF-κB p65 subunit, IL-1, and TNF, according to Western blot analysis. TGP treatment significantly suppressed TLR2 and TLR4 and MyD88 mRNA levels in all diabetic rats, according to real-time quantitative polymerase chain reaction analysis. In addition, macrophage infiltration was significantly increased in the kidneys of diabetic rats, but it was dose-dependently inhibited by TGP. These results implied that TGP protects several drug targets during the progression of DN by selectively inhibiting TLR activation (117).

Maharjan et al. (40) demonstrated that the renal tubules of STZ-induced diabetic rats express TLR2 and its endogenous ligands HMGB1 and HSP70 (40). They showed that the upregulation of TLR4 expression in infiltrating interstitial and renal tubular macrophages in human biopsies was attributable to DN (39). Moreover, TLR4 activation can lead to inflammation, tubular epithelial cell damage, and interstitial fibrosis in the progression of DN (120), and the results demonstrated that HG is involved in podocyte cell injury by activating the TLR2 and TLR4 signaling pathways.

Garibotto et al. (78) investigated TLR4 and TLR2 to initiate signaling to produce inflammatory cytokines and an inflammatory response that might lead to insulin resistance and progression of DN to ESRD in T2DM. Other authors have suggested that TLR activation stimulates the expression of several chemokine and inflammatory cytokines, such as TNF-α and CCL2 and IL-6, which are linked to the progression of DN (78).

TLR7

Experimental Studies

Yayi et al. (121) demonstrated that TLR7 expression is primarily restricted to renal tubular epithelial cells. Renal ischemia-reperfusion causes decreased renal function, pathological renal damage, and inflammatory factor release in diabetic rats.

Using HK-2 cell cultures, they discovered that inhibiting TLR7 increases the inflammatory reaction brought on by hypoxia-reoxygenation and HG levels, decreases apoptosis, and improves viability, all of which significantly desensitize HK-2 cells to the damage caused by glucose hypoxia-reoxygenation. This mechanism may prevent TLR7/MyD88/NF-κB signaling from achieving renal protection. This study demonstrated that HG levels increase the vulnerability of the kidney to severe ischemia-reperfusion injury. The experiments revealed that HG levels increase the production of inflammatory factors, worsen cell death, and decrease cell function. The results of a diabetes model showed that diabetes enhances the susceptibility to ischemic acute kidney injury (AKI) and suggests that a robust inflammatory response causes this susceptibility. TLR7 upregulation significantly improved renal damage in patients with diabetes after ischemic AKI. As a result, it was hypothesized that unusual TLR7 expression is linked to human renal tubular epithelial cells' susceptibility to hypoxia-reoxygenation and HG levels. Their findings indicated that high TLR7 expression is essential for renal injury vulnerability (122). Recent studies have shown elevated expression of specific inflammatory cytokines in diabetic kidneys, including IL-6, TNF-α, CCL2/MCP-1, C-X-C motif chemokine ligand 1 (CXCL1), and CXCl10/IP-10 (123,124). Renal ischemia in AKI animals led to the upregulation of several inflammatory cytokines and chemokine and the infiltration of some leukocytes into the kidneys, such as natural killer T-cells and T and B lymphocytes, including DCs, neutrophils, and macrophages (125).

The study of TLR7 in the lungs, liver, coronary arteries, and associated disorders has disclosed interesting results (126-128). According to Yayi et al. (121), TLR 7 suppression lessened acute renal ischemia/reperfusion injury in STZ-induced diabetic SD rats. TLR 7 is required for acute kidney ischemia/reperfusion injury in STZ-induced diabetic rats (121).

TLR7 primarily produces an inflammatory response in immune complex-mediated renal nephropathy, including lupus nephritis (122). TLRs may worsen renal damage in renal ischemia-reperfusion injury, ischemic AKI, renal fibrosis, antibody-mediated glomerulonephritis, and acute allograft rejection (39,41).

Clinical Studies

Huang et al. (122) experimented with TLR7 controls to enhance the vulnerability of patients to AKI. The mechanism of its pathogenesis is unknown. This study aimed to investigate the role of TLR7 and inflammation in ischemic AKI in patients with diabetes. Using a high-glucose hypoxia-reoxygenation model of human renal tubular epithelial cells (HK-2), we generated ischemia-reperfusion-induced AKI in diabetes. Hypoxia-reoxygenation was performed in groups with high and low glucose levels. Only modest cell damage, inflammation, and apoptosis were elicited in the low-glucose group. The high-glucose group, however, experienced substantial cell damage, inflammatory response, and apoptosis due

to a hypoxia-reoxygenation injury. TLR7 expression was more significant in the high- and low-glucose groups. Furthermore, the expression of TLR7 and related proteins was higher in the increased glucose group following reoxygenation after hypoxia. TLR7 inhibition provided excellent protection against ischemic injury in patients with diabetes. Basal TLR7 expression initiated protective signaling mechanisms against innate immunity against kidney injury when the immune system is in balance. Nonetheless, an imbalance in immune mechanisms at downstream factors and receptor levels can cause severe kidney damage (Figure 3) (122).

TLR9

Experimental Studies

Some studies have shown that TLR9 activity was responsible for systemic inflammation (129). Ito et al. (130) showed that a therapeutic dose of the C-C chemokine receptor type 2 antagonist INCB3344 significantly reduced inflammatory disease, dependently inhibiting macrophage invasion of organs, in rodent models. Mice were administered INCB3344 and C-C chemokine type 2 antagonists for 8 weeks. Decreased albuminuria, serum creatinine, TLR9 expression, and TNF-α production were detected. With the development of DN, TLR9 expression, ROS production, TNF-α production, and phagocytic activity were significantly increased. These results identified TLR9 as a factor responsible for the progression of DN and provided a novel strategy for treating the disease by targeting macrophages and their TLR9 expression via INCB3344 (130).

In DN, mitochondrial dysfunction and mitochondrial DNA (mtDNA) leakage are due to decreased levels of mtDNA superoxide dismutase 2 (SOD2 activates and stimulates TLR9, which is present in macrophages). In db/db mice, the proximal renal tubule cells produced more mtROS (131). TLR9 expression was enhanced in both macrophage types. L-carnitine treatment reduced mtROS production in proximal tubular cells and macrophages CD11b+low while improving SOD2 expression in the kidney, lowering circulating mtDNA content, and decreasing albuminuria (132). Furthermore, it inhibited macrophages infiltration into the kidneys and decreased TLR9 expression in macrophages, lessening tumor necrosis factor production in CD11b+high macrophages and ROS production in CD11b+low macrophages. The effects of L-carnitine on DN have shown its potential as a new treatment agent for DN caused by obesity (130).

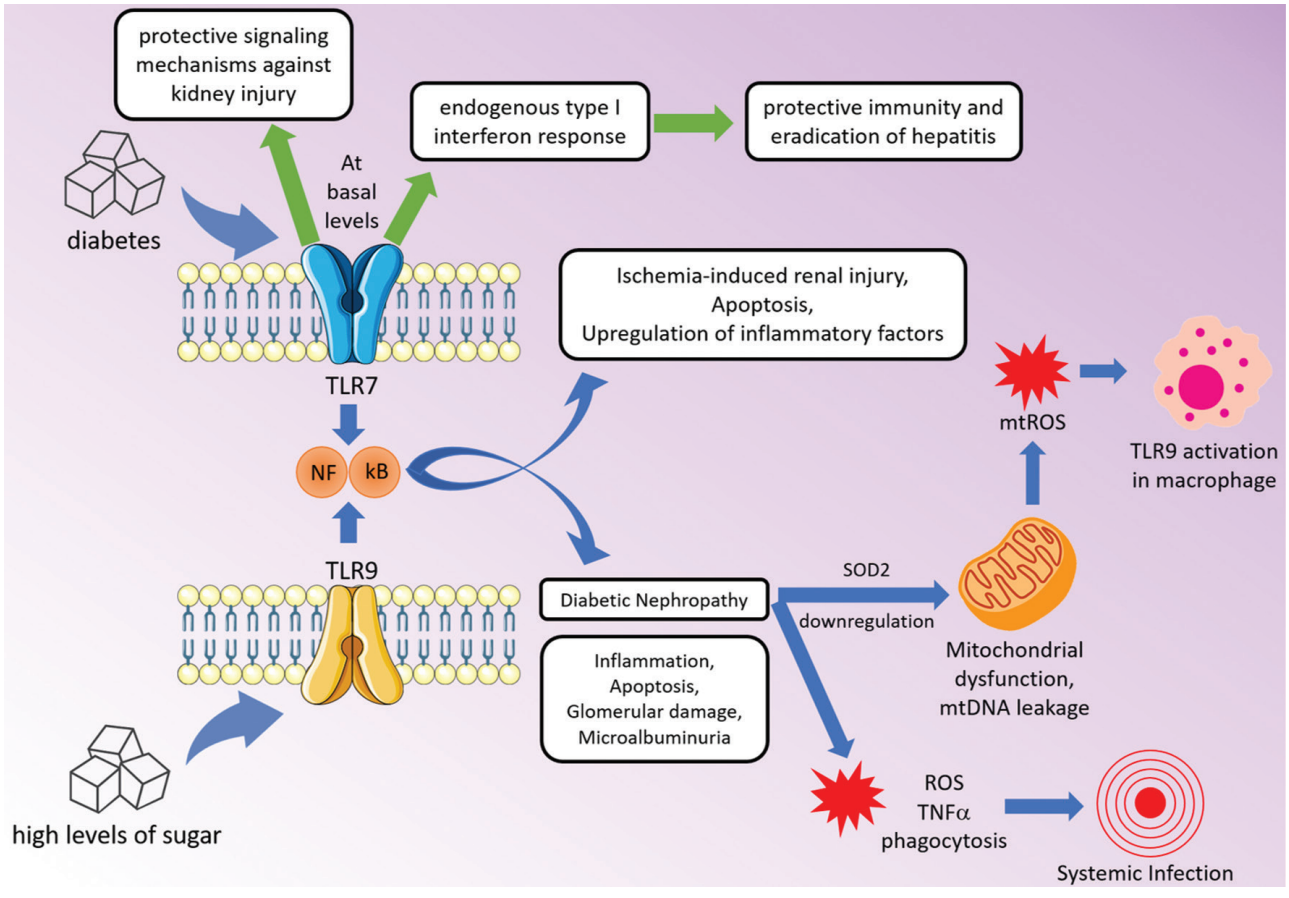

Figure 3. Stablished a summary of cellular mechanisms mediated by TLR7 and TLR9.
mtROS: Mitochondrial reactive oxygen species, TLR7: Tool-like receptor 7, TLR9: Tool-like receptor 9, ROS:Reactive oxygen species, TNF-α: Tumor necrosis factor alpha, NF: Nuclear factor, kB: Kappa B, SOD2: Superoxide dismutase-2

In large clinical trials of patients with DN, glycaemic control was shown to reduce early DN onset and progression to overt DN (133). Although the progression of DN cannot be defined once the disease is evident, extracellular vesicles can be induced by currently available treatments, including renin-angiotensin-aldosterone inhibitors, glycemic control, and blood pressure management (134). Additionally, by decreasing myocardial mtDNA damage and promoting mitochondrial biosynthesis, sodium-glucose cotransporter two inhibitors have been associated with enhanced cardiac metabolism and cardiac ATP generation (135) and could help protect kidney mitochondria. Mima reported in his studies that reasonable glycemic control might be the best preventive measure for DN, which develops despite diabetes treatment (136-138). Inhibitors of oxidative stress and inflammation can provide valuable therapeutic targets. The recovery of renal cells and renal function depends on the ATP-producing capacity of mitochondria (139). As a result, DKD treatment that targets the suppression of oxidative stress or improvement of mitochondrial homeostasis can be beneficial (132). Therefore, there is a need to develop therapies that target disease mechanisms in patients with advanced DN. Therapeutic approaches that can safely be added to current treatments include preventing macrophage infiltration into the kidney and controlling macrophage activity via mitochondrial protection in the kidney (132).

Shen et al. (140) determined the effects of the TLR9 and NF-κB/NLRP3 inflammasome on inflammation and cell death in DN. They investigated TLR9 expression levels in subjects with DN, the DN experimental model (db/db), and high-glucose-treated mouse mesangial cell strains (HG-treated MCs) as a DN cell model. In the PBMCs of patients with DN and in the kidneys of the DN experimental model (db/db) and DN cell model, TLR9 expression was highly expressed in comparison to controls at both the mRNA and protein levels, implying that high sugar levels result in TLR9 and have been linked to DN development. They performed an experimental TLR9 knockdown model (db/db) by injecting lentiviral shRNA-TLR9 into the tail vein for four weeks. They found that kidney and body weights, blood glucose levels, proteinuria, and the glomerular filtration marker serum creatinine (SCR) level were all significantly lower in the TLR9-depleted experimental model (db/db). These findings suggest that TLR9 is triggered by increased sugar levels and is associated with the emergence of DN (140).

TLR9 is an upstream component of the NF-κB-NLRP3 inflammasome pathway, which promotes inflammation in DN (141,142). IHC analysis revealed that the levels of NLRP3 inflammasome components, such as phospho-NF-κB, NLRP3, cysteinyl aspartate-specific proteinase-1, PYCARD, and IL-1 (140), were noticeably upregulated in the renal tissues of the experimental model (db/db). In contrast, the levels of these proteins were noticeably downregulated in the TLR9-NF-κB-NLRP3 inflammatory axis. Evaluating pro-inflammatory cytokines such as IL-6, IL-18, and TNF-α in the DN and TLR9-depleted cell models showed that the TLR9-NF-κB-NLRP3 inflammatory axis significantly controls inflammation in the DN cell model (110). Additionally, it was discovered that high-glucose treatment causes apoptosis in MCs (143,144). Through the activation of NF-κB, TLR9 knockdown prevented apoptosis in MCs exposed to HG. The activator betulinic acid completely reversed this effect (140). Examination of kidney histology by periodic acid-schiff (PAS) staining in the experimental DN (db/db) model showed that PAS-positive mesangial matrix areas were significantly increased but partially decreased by TLR9 knockdown (140). A lower glomerular matrix expansion index score in an experimental model of TLR9 deletion (db/db) suggests that renal pathological changes are inhibited by TLR9 knockdown. A typical manifestation of DN is increased levels of collagen IV and fibronectin (145), which were elevated in the glomerular mesangial regions of the DN experimental model (db/db). In contrast, lower levels were observed in the glomeruli of the TLR9-knockdown experimental model (db/db), indicating that during DN, TLR9 knockdown reduced fibronectin expression and suppressed collagen IV (140).

TLR2, TLR4, TLR5, TLR7, TLR8, and TLR11

Experimental Studies

Feng et al. (146) investigated the role of TLRs in diabetic renal lesions in a miniature pig model. They chose miniature pigs to make a diabetic model because they are more similar to humans in terms of kidney structure, metabolism, and immune function. Hence, they are ideal large-animal models for studying DN mechanisms. After establishing a diabetes model by feeding miniature pigs with high-sugar and high-fat diets for eight months, they examined pathological changes in diabetic kidneys and expression changes in TLR, downstream molecules, and endogenous TLR ligands (146). The DM group showed typical manifestations of early diabetes in the kidney tissues, such as marked glomerular hypertrophy and larger glomerular areas, compared with the control group (146). Unlike HMGB1, HSP70 expression was significantly upregulated in diabetic porcine kidneys (43,147,148). Furthermore, an increase in the expression of MyD88-dependent TLRs like TLR2, TLR4, TLR5, TLR7, TLR8, and TLR11, as well as their downstream signaling molecules MyD88 and phospho-IRAK-1 (activated IRAK-1), TLR3, and TLR4, and their downstream signaling molecule phosphor-IRF-3 (activated IRF-3) was detected. In addition,

NF-κB pathway molecules such as phosphor-inhibitory B kinase (phospho-IKK), phosphorylated inhibitor of NF B- (phospho-IB), nuclear factor B phosphorylated p65 subunit (NF-κBp65), and phospho-NF-κBp65 (p-p65) were significantly upregulated (140). Furthermore, the expressions of IL-6, MIP-2, MCP-1, CCL5, VCAM-1, and the macrophage marker CD68 were significantly increased, indicating macrophage infiltration in diabetic porcine kidneys (140). In DM models, metabolic substrates such as free fatty acids and HG directly or indirectly activate TLRs by stimulating cells to release endogenous TLR ligands such as HSP70, promote the downstream inflammatory response signaling pathway, and induce DM and its sequelae (43,147,148). This downstream inflammatory response signaling pathway activated the NFκ-B and IRF-3 transcription factors, which produce proinflammatory cytokines, inflammation, and autoimmune kidney disorders (149,150). These results suggest that extra nutrients and metabolites can cause abnormal immune responses that trigger inflammatory responses (149,150).

Discussion

DN is a microvascular consequence of diabetes and is also a leading cause of cardiovascular and chronic renal disorders (151). Inflammation is crucial for the pathophysiological development of DN. Network cytokines are essential for determining the direction of immune responses (152). As a crucial component of the immune response, TLRs are the only major transmembrane proteins in mammals that transmit antigen recognition data from the outside to the inside of the cell (153). TLR expression was noticeably higher in individuals with diabetes and renal failure than in the standard and other groups. TLRs are members of a superfamily of innate immune system receptors that can activate inflammatory response signaling pathways, including the NF-κB pathway, triggering and inducing acquired immunological responses (154,155). In addition to PAMPs, some endogenous molecules linked to cell damage, referred to as DAMPs, include nuclear DNA binding protein HMGB1, HSPs, and damaged mitochondrial DNA (156), can trigger inflammatory reactions by interacting with TLRs both intracellularly and extracellularly (157). Hence, the metabolic substrates linked to diabetes may directly engage TLRs or indirectly encourage the creation of endogenous TLR ligands, activating the downstream inflammatory response signaling cascade and causing the onset of diabetes and comorbidities (146).

TLRs 3, 7, 8, and 9 are found in internal vesicles (lysosomes and endosomes) and the endoplasmic reticulum, whereas TLRs 1, 2, 4, 5, 6, and 10 are present on the cell membrane (158). Functional and structural alterations may be influenced by the activation of TLRs by PAMPs and DAMPs (159). TLR2 can detect PAMPs from different infections (viruses, bacteria, fungi, and parasites). Moreover, TLR2 and TLR4 may detect DAMPs generated during inflammation (47,158). TLR9 identifies only bacteria and TLR3 only viruses (158). TLRs identify these molecular messages by recruiting particular adaptor molecules such as TRAM (TRIF-related adaptor molecule), TIRAP (TIR domain-containing adaptor protein), MyD88 (myeloid differentiation factor 88), and TRIF (TIR domain-containing adaptor-inducing interferon-β) resulting in the activation of the transcription factor nuclear factor-kappa B (NF-κB). The activated NF-κB then localizes to the nucleus and stimulates the production of the proinflammatory mediators IL-1 (pro-IL-1) and IL-18 (pro-IL-18) (146,158,160-162). According to Wolf et al. (147), the expression of MyD88-dependent TLR2, TLR4, TLR5, TLR7, TLR8, and TLR11, MyD88, and activated IRAK-1 (phospho-IRAK-1) were shown to be highly upregulated in the micro pig diabetic model. Also, their research revealed that diabetic kidneys had notably higher expression levels of MyD88-independent TLR3 and TLR4 and activated IRF-3. These results indicated that MyD88-dependent and MyD88-independent TLRs were markedly upregulated in diabetic kidneys and might further stimulate downstream signaling pathway components (146). Although it is known that TLR2 and TLR4 are linked to DN, the function of all TLRs in diabetic kidney lesions is unknown.

TLR2 is a critical element of TLRs, and its activity has been extensively explored. Zhang et al. (58) discovered that TGP improved the disruption of e-cad in DNs via immunohistochemical labeling. The most typical marker for identifying myofibroblasts is -SMA. Their investigation indicated that in DN, -SMA immunostaining was observed in some glomeruli, tubules, and the interstitium, whereas in normal rats, it was exclusively expressed in the vascular walls. The use of TGP had a depressing effect on DN expression. These findings revealed that TGP might prevent the trans-differentiation of renal tubular epithelial-mesenchymal cells in DN. Their most recent study showed that NF-κB activation was associated with TLR2 expression in DN, which increased macrophage infiltration. Treatment with TGP may decrease the numbers of ED-1+ and ED-1+ TLR2+ cells and expression of TLR2 and NF-κB in DN (58). Nevertheless, prior research has demonstrated that renal illness is a dynamic and complex mechanism controlled by a wide range of cellular elements and cytokines, including the action of numerous genes and regulatory variables in renal injury (163,164).

Consequently, effective treatment of renal damage requires a thorough understanding of the unique molecular regulatory network of SOCS1. In mice, TLR2 dramatically

lowers serum cytokine levels, leading to oliguria and renal histological damage (165). This suggests that genes or proteins that can control this pathway could serve as potential targets for the treatment of renal disorders. Elevated SOCS1 levels shielded kidneys by blocking the TLR signaling pathway. The essential pathway genes TLR2 and MyD88 and the indicator INF were markedly downregulated in the SOCS1 overexpression group as renal illness progressed.

Additionally, the levels of TLR2 and MyD88 expression were noticeably decreased in the SOCS1 overexpression group, suggesting that SOCS1 overexpression can aid in the recovery of renal injury in conditions affecting the kidneys. This finding was in line with the research of Mudaliar et al. (41). In conclusion, our findings revealed that SOCS1 decreased the TLR signaling pathway, which affected kidney damage in renal disorders (61).

TLR4 is a component of the TLR family and is intimately connected to the pathophysiology of numerous illnesses, including diabetes, Alzheimer's disease, and various malignancies (166,167). TLR4 has also been identified as a detrimental signaling pathway in renal fibrosis and DN (78,168). Furthermore, TLRs, such as TLR4, activate various downstream regulatory pathways or molecules, like NF-κB and interferon regulatory factors (45,169). In STZ-induced DN mice, TLR4 deletion reduced renal hypertrophy, renal damage, and inflammatory and fibrotic responses. Moreover, HG stimulation activated TLR4 signaling in podocyte and tubular epithelial cells, activating NF-κB signaling and increasing inflammatory and fibrogenic protein levels (120). Moreover, deletion of TLR4 reduced tubular damage and prevented the onset of renal fibrosis by deactivating proinflammatory signals, such as NF-κB in cyclosporine nephrotoxicity (170). Zhang et al. (68) showed that miR-124 prevented the activation of the TLR4/NF-κB messaging pathway in HG-treated HK-2 cells. Furthermore, by inhibiting the NF-κB signaling pathway using its blocker, PDTC decreased the expression of COLIV and FN in HK-2 cells exposed to HG. Baicalin reduced renal fibrosis in STZ-induced DN mice and HG-treated HK-2 cells by upregulating miR-124 expression and suppressing TLR4/NF-κB pathway activation. These findings offer a novel understanding of the molecular basis of baicalin and potential targets for treating DN and renal fibrosis (68). Moreover, in the Zhu et al. (171) study, the rat model of DN was successfully created to investigate whether miR-195 targeted TLR4 and blocked the NF-κB signaling pathway and its effect on DN rats. MiR-195 expression in normal tissues was significantly higher than that in DN tissues. Research has demonstrated a negative connection (r^2=0.4836, p=0.0007) among the expressions of miR-195 and TLR4. It has been demonstrated that miR-195 might negatively influence TLR4 protein expression (171).

Compared with nephrotic patients without renal failure, nephrotic patients with renal failure have significantly higher expression of TLR2 and TLR4. Furthermore, TNF-, IL-6, and IFN- expression was enhanced along with TLR2 and TLR4 activity, worsening insulin resistance associated with inflammation. These alterations were more evident in individuals with diabetes and end-stage kidney disorders and renal failure (6). According to Wang et al.'s study (116), TLR2 and TLR4 are crucial in podocyte damage and the inflammatory response brought on by HG. In addition, HUC-MSCs can protect podocyte against HGs by controlling the TLR signaling pathway, most likely through the secretion of soluble hepatocyte growth factor (HGF). Patients with DN may experience significant advantages with MSC-based therapy.

One of the fundamental mechanisms by which the TLR group regulates the inflammatory response is TLR7. Previous research using a murine lupus model showed that TLR7 may exert contrasting inflammatory and regulatory effects (172). The information reported in this study also indicates that TLR7 stimulation can quicken autoimmune diabetes-induced acute renal I/R damage in SD rats. According to prior research, it can be concluded that TLR7 and its downstream effector MyD88/NF-κB were considerably elevated at protein levels in the kidneys of I/R diabetic rats compared with the diabetic group. MyD88 and NF-κB protein levels appear to be less activated when TLR7 expression is reduced in the cytoplasm. These results imply that renal I/R in T1DM activated TLR7, targeting renal tubular epithelial cells, among other targets. Ultimately, the inflammatory response observed in renal tissue could be caused by excessive activation of TLR7/MyD88/NF-κB-dependent innate immunity under renal I/R in patients with T1DM and may be involved in the onset and development of renal I/R injury in patients with diabetes. Future investigations will clarify the precise pathways involved. The TLR7 signaling pathway was inhibited in chloroquine-treated samples, accompanied by the downregulation of MyD88 and NF-κB. Renal impairment in T1DM is worsened by TLR7, which is released in response to ischemia. These findings indicate that TLR7 plays a significant role in diabetic I/R renal damage (121).

Furthermore, the findings of Huang et al. (122) supported the notion that diabetes elevates ischemic AKI susceptibility, which is a consequence of a significant inflammatory reaction. They discovered that overexpression of TLR7 significantly worsens kidney damage in diabetes following ischemic AKI, and they put forth the hypothesis that aberrant TLR7 expression is linked to susceptibility

to elevated glucose and hypoxia-reoxygenation in human tubular epithelial cells of the kidney. Their research showed that TLR7 overexpression significantly contributes to renal damage susceptibility. In conclusion, their research suggested that the increased inflammatory reaction and ischemia susceptibility of diabetic kidneys may be caused by the overexpression of TLR7 in the kidney (122).

Comparatively to the renal tissues from experimental mice (db/db), HG-treated MCs, PBMCs, and renal tissues from patients with DN all had higher TLR9 expression rates than the corresponding controls. Via the NLRP3 inflammasome, inhibition of TLR9 expression directly regulated NF-κB and lowered the expression of inflammatory molecules *in vitro* and *in vivo*. In DN mice, TLR9 is involved in the growth of fibrosis and microalbuminuria (53). These results provide a unique theory for the NF-κB-mediated constitutive activation of the NLRP3 inflammatory pathway in DN. In contrast, after *TLR9* gene suppression, the increased expression of type IV collagen and fibronectin in the experimental db/db mice was suppressed compared with that in the NC group (53).

Moreover, body/kidney weight and SCR expression were drastically altered after deletion of TLR9. These results imply that TLR9 plays a crucial role in the later phases of DN in experimental mice (db/db). According to research by Shen et al. (140), TLR9 modulates NF-B upstream. TLR9 suppression and BA use in HG-treated MCs demonstrated that inhibition of IκB phosphorylation and decreased nuclear NF-κB localization influenced inflammation and controlled apoptosis. TLR9 reduction may cause kidney protective activities by inhibiting NLRP3 inflammasome activation, and the synthesis of IL-1 and cysteinyl aspartate specific proteinase-1 could be affected by TLR9's regulatory function in NF-κB activation (53,142). Overall, these findings imply that the TLR9-related NLRP3 inflammasome may be a potential therapeutic target for DN.

In addition, the expression of endogenous TLR ligands and multiple TLRs was considerably upregulated in diabetic miniature swine kidneys, which may then activate NF-κB and IRF-3 signaling via MyD88-dependent and MyD88-independent mechanisms and other processes to induce metabolic inflammation in renal tissues, finally leading to the occurrence and progression of diabetic renal injury (146). Our study is primarily oriented toward animal findings of the topic; there needs to be more human study data presented in our review of the subject; we consider this as a shortcoming of our study, and we aspire future research to be more focused on human findings (Figure 4).

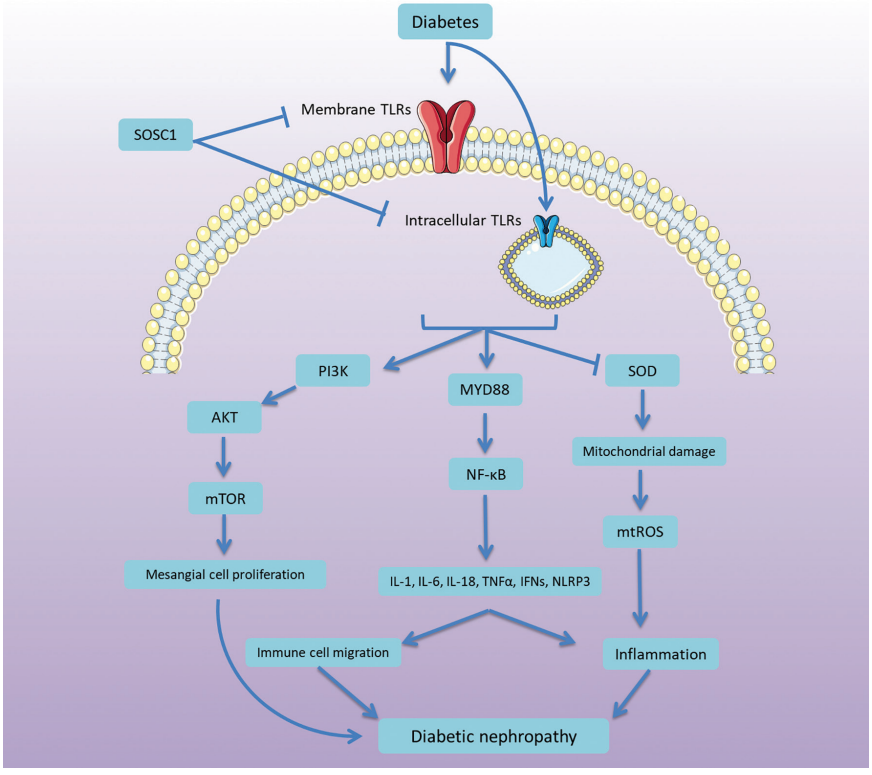

Figure 4. Summarizes TLR-mediated cellular pathways leading to diabetic nephropathy.
mtROS: Mitochondrial reactive oxygen species, TNF-α: Tumor necrosis factor alpha, NF-kB: Nuclear factor kappa B, mTOR: Mechanistic target of rapamycin PI3K: Phosphatidylinositol-3-kinase, AKT: Protein kinase B, IL: Interleukin, NLRP3: Nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3, IFNs: Immune interferons

Conclusion

The findings of this review highlight the role of TLRs in kidney diseases, particularly DN. The metabolic inflammation activated by TLRs may be significant in diabetic renal injuries, as suggested by several *in vitro*, *in vivo*, and clinical trials. The functions of TLR4 and TLR2 in cultivated tubular cells and podocyte have been the subject of numerous studies. The expression levels of TLR2 and TLR4 are significantly higher in patients with renal failure who have nephrotic diabetes than in those without renal failure. Additionally, greater TNF, IL-6, and IFN expressions and increased insulin resistance in response to inflammation strongly correlate with TLR2 and TLR4 activity. TLR2 and TLR4 play critical roles in podocyte injury and inflammation caused by HG, and HUC-MSCs can protect podocyte by regulating the TLR signaling pathway, likely through the secretion of soluble HGF. In addition, studies have been conducted for each of these two TLRs and showed that SOCS1 protects against the progression of DN by mediating the TLR2-MyD88 pathway to alleviate the inflammatory response. Baicalin limited renal fibrosis in STZ-induced DN mice and HG-treated HK-2 cells by upregulating miR-124 expression and inhibiting TLR4/NF-κB pathway activation. To prevent or delay DN in patients with T2DM, TLR2 and TLR4 may be useful therapeutic targets. Numerous studies have shown that TLR 7 may contribute to the etiology of kidney damage caused by renal ischemia/reperfusion in T1DM. The enhanced inflammatory response and diabetic kidney susceptibility to ischemia may be addressed by TLR7 overexpression in the kidney, and TLR7 inhibition reduces the damage caused by diabetic renal ischemia/reperfusion. Similarly, In HG-treated MCs and mice (db/db) with DN, downregulating TLR9 expression inhibits the production of proteins linked to inflammation and apoptosis via the NLRP3 and NF-κB NLRP3inflammasome pathways. TLR2 and TLR4 may, therefore, represent attractive therapeutic targets for halting or delaying DN in people with T2DM.

Authorship Contributions

Concept: M.S.G.C., Y.T., Design: M.S.G.C., Y.T., Av.A., M.A., H.A., N.G., A.A., I.A., S.K., A.An., M.P., Z.M., E.F., G.E., S.A.M., M.S.F., N.D., P.A.D., Data Collection or Processing: N.G., Z.M., Literature Search: M.S.G.C., Y.T., A.A., M.A., H.A., N.G., A.A., I.A., S.K., A.An., M.P., Z.M., E.F., G.E., S.A.M., M.S.F., N.D., P.A.D., Writing: M.S.G.C., Y.T., A.A., M.A., H.A., N.G., A.A., I.A., S.K., A.An., M.P., Z.M., E.F., G.E., S.A.M., M.S.F., N.D., P.A.D.

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