Advances in understanding the innate immune-associated diabetic kidney disease

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Abstract
Millions of human deaths occur annually due to chronic kidney disease, caused by diabetic kidney disease (DKD). Despite having effective drugs controlling the hyperglycemia and high blood pressure, the incidence of DKD is increasing, which indicates the need for the development of novel therapies to control DKD. In this article, we discussed the recent advancements in the basic innate immune mechanisms in renal tissues triggered under the diabetes environment, leading to the pathogenesis and progression of DKD. We also summarized the currently available innate immune molecules-targeting therapies tested against DKD in clinical and preclinical settings, and highlighted additional drug targets that could potentially be employed for the treatment of DKD. The improved understanding of the disease pathogenesis may open avenues for the development of novel therapies to rein in DKD, which consequently, can reduce morbidity and mortality in humans in the future.

KEYWORDS
diabetic kidney disease, innate immunity, therapy

Abbreviations: AGEs, advanced glycation end products; ASC, apoptosis-associated speck-like protein containing a CARD; CCL, C-C motif chemokine ligand; CCR2, C-C motif chemokine receptor 2; DAMPs, damage-associated molecular patterns; DKD, diabetic kidney disease; ELM01, engulfment and cell motility protein 1; eNOS, endothelial nitric oxide synthase; ERK, Extracellular signal-regulated kinase; GWAS, genome-wide association study; HMGB1, high mobility group protein B1; HSPs, heat-shock proteins; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IKKβ, inhibitor of nuclear factor kappa-B kinase subunit beta; IL, interleukin; IRAK1, interleukin-1 receptor-associated kinase 1; IRFs, interferon regulatory factors; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; JAK, Janus tyrosine kinase; MAC, membrane attack complex; MAPKs, MAP kinases; MASPs, MBL-associated serine proteases; MBL, mannose-binding lectin; MMP, matrix metalloproteinase; MyD88, Myeloid differentiation factor 88; NF-κB, nuclear factor kappa-B; NLR, nucleotide-binding oligomerization domain-like receptor; NLRP3, NLR family pyrin domain containing 3; NOD, nucleotide-binding oligomerization domain-like receptor; NLRP3, NLR family pyrin domain containing 3; NOD, nucleotide-binding oligomerization domain-like receptor; NLRP3, NLR family pyrin domain containing 3; NOD, nucleotide-binding oligomerization domain-like receptor; NLRP3, NLR family pyrin domain containing 3; PAMPs, pathogen-associated molecular patterns; PPAR, pattern recognition receptors; ROS, reactive oxygen species; SGLT2, sodium-glucose cotransporter-2; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; STZ, streptozotocin; TGF, transforming growth factor; TIRAP, TIR Domain Containing Adaptor Protein; TLR, toll-like receptor; TNF, tumor necrosis factor; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain containing adapter-inducing interferon-beta; TYK2, tyrosine kinase 2; VCAM-1, vascular cell adhesion molecule 1.

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Diabetic kidney disease (DKD), also called diabetic nephropathy, is a serious kidney-related complication of diabetes mellitus. As a result of the growing incidence of diabetes mellitus, DKD is considered as one of the major causes of chronic kidney disease around the globe with significant morbidity and mortality. According to the recent World Health Organization report, the global prevalence of diabetes mellitus is ~422 million people, particularly in low- or middle-income countries, with roughly 1.6 million deaths each year. A systemic review of 540 data resources estimated that the number of people with diabetes is expected to be increased to ~642 million by 2040. The global mortality rate of chronic kidney disease increased by 41.5% from 1990 to 2017. Chronic kidney disease resulted in 35.8 million disability-adjusted life-years in 2017, with DKD accounting for almost a third of disability-adjusted life-years.

DKD can be characterized by both changes in the function and the structure of the kidney. Functionally, there is the aberrant release of albumin in the urine (albuminuria) and the loss of glomerular filtration rate. Structurally, there is glomerular mesangial expansion, the diffuse thickening of the glomerular basement membrane, nodular glomerulosclerosis, podocytes loss, the disruption of endothelial cells, tubular hypertrophy (at early stages), tubular atrophy (at late stages), interstitial fibrosis, arteriolar hyalinosis, and the infiltration of immune cells. In a diabetic environment, the alterations in metabolic and hemodynamic processes initiate innate immune signaling cascades in tubular epithelial cells, podocytes, and mesangial cells, which mediate the cellular response via activation of the innate immune molecules and transcription factors, resulting in the onset of inflammatory responses. Of these molecules, some act as chemotactic molecules, and thus, promote the recruitment of immune cells (mainly macrophages) at the site of inflammation, which contributes to inflammation, oxidative stress, and fibrosis through releasing inflammatory mediators, reactive oxygen species, and antiangiogenic factors.

Control of classical deleterious factors in patients with diabetes, such as hyperglycemia and elevated blood pressure has been demonstrated to be critical in preventing the development and progression of DKD. Despite the establishment of these therapeutic strategies, the current treatment of DKD is suboptimal. Therefore, the novel perspectives of DKD as a disease with the involvement of inflammatory processes provide a rationale for the development of novel therapeutic options based on targeting the inflammatory mediators.

In the present article, we reviewed recent advancements in understanding the role of innate immunity in the development of DKD, and discussed the current status, benefits, and limitations of currently available therapeutic options against DKD. Based on available knowledge, research gaps have also been highlighted that might be crucial to understand the disease pathology and to develop novel therapies to treat DKD.
actin and impairing the E-cadherin expression in these cells.\textsuperscript{17} Treatment of cultured tubular and mesangial cells with high glucose-induced expression of HMGB1, which in turn augments NF-κB activation, inflammation, and oxidative stress in these cells.\textsuperscript{18,19} The albumin-induced expression of another DAMP, HSP70, in tubular cells has been associated with enhanced TLR4 singling cascade and the ultimate production of inflammatory cytokines.\textsuperscript{20} Alternatively, the long-noncoding RNA, Gm6135, via sponging microRNA-203, regulates the hyperglycemia-induced TLR4 expression in mesangial cells, resulting in the induction of DKD.\textsuperscript{21}

Several experimental animal studies also suggested the participation of TLR2 and TLR4 in inducing DKD. Hyperglycemia-induced activation of TLR2 and TLR4 in mice kidney tissues was found to associate with renal injury, inflammation, interstitial fibrosis, podocytopathy, and albuminuria, whereas the depletion of these TLRs in mice inhibited the onset of such effects.\textsuperscript{22-24} In a miniature pig model of DKD, the expression or activity of MyD88-dependent TLRs (TLR3/4), and their downstream signaling molecules, including MyD88, p-IRAK1, p-IRF3, p-IKKβ, p-IκBα, NF-κB, p-NF-κB, IL-6, C-C motif chemokine ligand (CCL)2, CCL5, macrophage inflammatory protein 2, and vascular cell adhesion molecule 1 (VCAM-1), was observed to be significantly increased in pig kidney tissues.\textsuperscript{25} Overall, these studies indicate that inflammatory responses stimulated by TLRs play a decisive role in the progression of DKD.

### 2.2 Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs)

NLRs are cytosolic PPRs that initiate innate immune response upon recognition of cytosolic PAMPs and DAMPs. NLRs can be classified into subfamilies based on either structural organization (NLRA/B/C/P) or functional characteristics (signaling transduction, inflammasome assembly, autophagy, and transcription activation). Among the NLRs, NOD2 (a member of the NLRC subfamily that transduces
signals) and NLR family pyrin domain containing 3 (NLRP3, a member of the NLRP subfamily that oligomerizes to form inflammasomes) are well-known owing to their role in the development of several diseases, such as liver diseases, cancers, and kidney diseases. Studies have provided the NOD2- or NLRs-mediated mechanisms of DKD (Figure 1, panel B).

A positive correlation of NOD2 and DKD has been determined with an increase in NOD2 expression in the mice and human patients suffering from severe DKD. Diabetic stimuli, including hyperglycemia, advanced glycation end products (AGEs), transforming growth factor (TGF)-β1, and tumor necrosis factor (TNF)-α, have been shown to induce NOD2 expression in cultured podocytes, whereas silencing of NOD2 protected podocytes from damage, and reduced inflammatory cytokines production, albuminuria, and mesangial cell expansion in vivo.

Accumulating evidence also suggests that mesangial cells, podocytes, tubular epithelial cells, and glomerular endothelial cells exhibit expression and activation of inflammasomes. Several diabetic stimuli such as hyperglycemia, hyperlipidemia, hyperuricemia, AEGs, thioredoxin-interacting protein, ATP-sensitive P2X purinoreceptor 4, and mitochondrial ROS were associated with increased NLRP3 inflammasome activity and stimulation of cytokines production in renal tissues. Examination of renal tissues isolated from diabetic human and rodents have revealed an enhanced expression of NLRP3 components (NLRP3 and caspase-1) associated with rampant activation of IL-1β and IL-18, leading to podocyte damage, mesangial cells accumulation, and albuminuria. Inhibition of NLRP3 and caspase-1 in a mouse model of DKD protected mice from diabetic-mediated renal damage. The consequences of over-activation of NLRP3 inflammasomes in cultured human renal proximal tubular cells are shown to reduce hyperglycemia-mediated epithelial-mesenchymal transition and dephosphorylation of signaling molecules that regulate inflammatory responses such as ERK1/2, SMAD3, and MAPK p38. The levels of a mitochondria-linked autophagy receptor, optineurin, has been observed to downregulate in tubular epithelial cells of patients suffering from DKD and was negatively correlated with NLRP3 activation, caspase-1 cleavage, and the release of IL-1β and IL-18. The increased activity of inflammasomes NLRc4 and NLRc5 has also been shown to associate with the induction of DKD. The diabetic mice deficient in NLRc4 abolished DKD progression with reduced blood glucose, albuminuria, and IL-1β production through abrogating the NF-κB and MAPK pathways. Similarly, the deficiency of NLRc5 in diabetic mice, by alleviating the NF-κB and TGF-β/SMAD signaling cascades, caused less diabetic kidney injury, as assessed by reduced levels of albuminuria, fibronectin, collagen IV, and macrophage infiltration. The roles of other inflammasomes in DKD is currently unknown.

### 2.3 Inflammatory mediators

Activation of TLRs and NLRs triggers the release of inflammatory mediators (ie, cytokines, chemokines, and cell adhesion molecules) predominantly via the transcription factor NF-κB. Increased expression of NF-κB in renal tissues of humans and mice with diabetes and renal macropathies strongly correlated with the parameters of renal damage. Phosphorylation of NF-κB p65 subunit in tubular and interstitial cells exacerbated inflammation and apoptosis, whereas the transgenic mice expressing the super-repressor IκBα in renal cells remained resistant to p65-mediated deleterious effect on kidney tissues. The cytokine TNF-like weak inducer of apoptosis-dependent activation of noncanonical NF-κB pathway in tubular cells, as determined by the nuclear translocation of NF-κB RelB and p52 subunits, increased kidney injury. In contrast, NF-κB can also exhibit anti-inflammatory activities and prevent renal damage. The pre-stimulation of NF-κB by lipopolysaccharide in mice inhibited renal injury, which was linked with the hypoxia-inducible factor-2α-regulated nitric oxide production in renal tissues. The NF-κB-depleted mice showed increased glomerular injury, proteinuria, and inflammatory cytokine production during acute, but not chronic, renal injury.

Several inflammatory cytokines and chemokines such as TNF-α, IL-1, IL-6, IL-18, CCL2, and CCL5 have been found to be upregulated in kidney tissues and are associated with several renal effects, including enhanced vascular endothelial permeability, increased mesangial cell proliferation, affect extracellular matrix synthesis, and increased albuminuria. Studies have also suggested the implication of TNF-α in inducing the cellular apoptosis and necrosis pathways, the destabilization of the intraglomerular hemodynamics affecting the glomerular filtration rate, and the induction of NADPH-mediated cellular oxidative stress leading to the production of ROS that eventually causes renal injury in diabetic human and mice. Among the cell adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) and VCAM-1 are the crucial ones associated with the development and progression of DKD. Elevated levels of ICAM-1 have been observed in experimental and clinical studies, and the inhibition of ICAM-1 in mice led to amelioration of ICAM-1-induced hallmark features of DKD such as glomerular hypertrophy and albuminuria. However, a recent study showed that plasma ICAM-1 level was not independently associated with the prevalence of DKD in Asians suffering from diabetes. Akin to ICAM-1, the circulating levels of VCAM-1 have also been found to rise in patients with diabetes, which may
occur due to injured tubular and glomerular endothelium or reduced renal clearance of this adhesion molecule.

In addition to directly developing the DKD, these inflammatory mediators are involved in the recruitment of immune and inflammatory cells from blood to renal tissues, which aggravate the renal injury by intensifying the inflammatory response.\(^5^8\) CCL2 from renal tissues is engaged in the direction of macrophages migration into the diabetic kidney.\(^6^9\) The production of ROS increases the expression of ICAM-1 and VCAM-1 on the surface of renal endothelial cells, which recruit leukocytes and macrophages to the site of inflammation and assist in the adhesion of these cells to endothelium and mediates subsequent transmigration.\(^7^0,7^1\) The inflammatory mediators-directed recruitment of macrophages in kidney tissues is positively correlated with the glomerular and tubular damage, increased plasma creatinine level, renal fibrosis, and expression of additional macrophages-derived cytokines and chemokines.\(^5^8,7^2\) The macrophage-derived TNF-α and CCL2 are found to be associated with altered glomerular hemodynamics, tubulointerstitial damage, glomerular hypertrophy, and albuminuria.\(^7^3,7^4\) The diphtheria toxin-induced ablation of macrophages in diabetic mice resulted in diminished albuminuria and ameliorated renal histopathological lesions.\(^5^7\) Furthermore, macrophages exhibit strong phenotypic plasticity, and the imbalance of M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotype contributes to DKD. The inhibition of cyclooxygenase-2 in macrophages conferred M1 phenotype and diabetic mice depleted with cyclooxygenase-2 exhibited enhanced DKD as determined by increased albuminuria and renal fibrosis with fewer renal M2 macrophages.\(^7^5\) Silencing of TGF-β-activated kinase 1 and triggering receptor expressed on myeloid cell 1 impeded polarization of macrophages into M1 phenotype, which conferred reduced renal inflammation in vitro and in vivo.\(^7^6,7^7\) Mesangial stem cells alleviated inflammatory response and ameliorated renal injuries in diabetic mice by eliciting M2 macrophages via the stimulation of the transcription factor EB and restoration of lysosomal activity and autophagy in macrophages.\(^7^8\) The treatment of diabetic mice with fasudil decreased M1/M2 macrophage ratio and increased anti-inflammatory cytokines production in the kidney, which ultimately attenuated renal injury.\(^7^9\) Hence, the role of macrophage phenotypes in DKD warrants further investigation into macrophages switch and its role in inflammation.

Variants in genes regulating the inflammatory status can also affect the incidence and progression of DKD. In a recent genome-wide association study (GWAS), the genotyping of 722 Spanish adults identified the single nucleotide variants of IL-1A (rs17561), IL-4 (rs2070874), IL-6 (rs1800797), and ICAM-1 (rs5498) genes that showed association with biochemical parameters of DKD, that is, glomerular filtration rate, phosphorus, and parathyroid hormone.\(^8^0\) The same variant of IL-4 and IL-6 genes were also associated with kidney function in the Japanese population.\(^8^1\) In addition, GWAS variants in some other inflammation regulatory genes such as engulfment and cell motility protein 1 (ELMO1), TGF-β, TNF-α, CCR5, IL-1β, IL-8, IL-10, IL-18, MMP9, and TNF receptor-associated factor 6 are linked with the pathogenesis of DKD,\(^8^2,8^3\) and some of these variants differ across populations. For instance, ELMO1-rs741301, ELMO1-rs10951509, and CCR5-rs1799987 in Chinese, ELMO1-intron 18+9170 in Japanese, TGF-β-rs1800470 in Chinese, Egyptians, and Mexicans, TGF-β-rs1800471 in Mexicans, and IL-8-rs4073 in Indians.\(^8^4,8^5\) More studies are needed to determine how these inflammatory genes’ variants influence the outcome of DKD.

### 2.4 Activation of the complement system

The cellular complement system is known to engage three pathways: classical, lectin, and alternative. The classical pathway is stimulated through the detection of antibodies by the C1 complex (C1q, C1r, and C1s), which leads to the cleavage of the components C2 and C4. The lectin pathway is triggered upon sensing of pathogen-derived carbohydrates and N-acetylglucosamine by mannose-binding lectin (MBL) and ficolins, respectively, followed by the activation of MBL-associated serine proteases (MASPs) that in turn cleave C2 and C4. The activation of the alternative pathway occurs via spontaneous stimulation of the component C3b. All three pathways result in the cleavage of the component C3 and subsequent formation of the C5b-9 complex (membrane attack complex, MAC).\(^8^6\)

Preclinical and clinical studies have confirmed the involvement of the complement system in inducing DKD primarily through two pathophysiological mechanisms: (a) hyperglycemia-induced glycation of cell surface proteins that over-activates lectin pathway and (b) hyperglycemia-induced glycation of complement regulatory proteins that results in the perturbation of their functions. As a consequence, both these mechanisms cause aberrant stimulation of complement pathways which favors the complement auto-attack through the excessive accumulation of MAC.\(^8^7,8^9\) Accumulating evidence suggests a correlation between the complement system and the onset of DKD (Figure 1, panel C). The components, C1q, C3c, C5a, and C7, have been observed to be abnormally accumulated in kidney tissues of patients with diabetes and induced severe kidney damage.\(^9^0,9^2\) The global RNA profiling of diabetic patient-derived kidney tissues revealed the enrichment of the complement system’s components that exhibit altered expressions upon diabetes. Among all the complement components, the expression of one specific component, C7, was observed to be significantly elevated in diabetic kidney tissues, when compared with the healthy human kidney tissue samples.\(^9^1\) In addition, the analysis of urine samples of
patients with diabetes has shown the excessive secretion of complement activation factors such as factor H-related protein 2, C3a, C5a, and C5b-9 that were positively correlated to the disease progression and increased mortality rate. Overall, these studies confirm the involvement of the complement system in DKD.

### 2.5 Janus tyrosine kinase-signaling transducer and activator of transcription (JAK-STAT) signaling

JAK and STAT proteins transduce signals from a variety of extracellular cytokines or chemokines receptors. Upon detecting the ligands, these membrane receptors recruit pairs of JAK proteins to the intracellular domain of receptors and activate them through autophosphorylation. The activated JAKs consequently induce the phosphorylation of the intracellular domains of the receptors which then act as the binding site for the STAT proteins. Once STATs are phosphorylated by JAKs, they undergo homo- or hetero-dimerization, followed by translocation from the cytoplasm into the nucleus to stimulate the transcription of target genes. In mammalian cells, there are four types of JAK (JAK1/2/3 and TYK2) and seven types of STAT (STAT1/2/3/4/5a/5b/6) members. JAKs and STATs are among the pivotal proteins involved in the regulation of several biological processes such as development, proliferation, and differentiation.

JAK and STAT proteins are also known to mediate innate immune responses in renal cells, including tubular cells, podocytes, and mesangial cells (Figure 1, panel D). Increased expression and activity of JAK1/2/3 and STAT1/3 is associated with the onset of DKD, whereas the reduction of JAK1/2 and STAT3 appears to ameliorate the disease outcome. A genome-wide transcriptomic analysis of human subjects with DKD revealed the elevated expression of JAK1/2 and STAT1/3, when compared with the control subjects. Mapping the canonical and metabolic transcriptional networks of DKD shared between human and mice indicated the JAK-STAT pathway as a canonical pathway with the highest degree of overlap between both species.

In the diabetic milieu, the presence of AEGs and the activation of MAPK can enhance the acetylation and phosphorylation status of STAT3 protein in mouse and human diabetic kidney tissues, leading to the increased transcriptional activity of STAT3. Silencing of STAT3 activity in mice reduced inflammatory response and abnormal matrix synthesis during the early stage of DKD. Ectopic expression of the suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in diabetic mice, reduced STAT1 and STAT3 activity, respectively, and abrogated inflammatory response, which contributed to the attenuated renal damage and improved renal function in treated mice. The role of other JAKs and STATs in the progression of DKD is currently unknown, hence, future studies in this research area may reveal a novel pathological mechanism of DKD.

### 3 THERAPEUTIC INTERVENTIONS TO TREAT DKD

#### 3.1 TLR signaling inhibitors

With the advancement in understanding the role of TLRs in inducing DKD, several preclinical studies have suggested a therapeutic potential of TLRs antagonists in attenuating the DKD and thereby providing the renoprotective effect (Figure 2, panel A). A multi-TLRs (TLR2/4/6)-specific antagonist, viz., (S, R)-3-phenyl-4,5-dihydro-5-isoxazole acetic acid (GIT-27), by acting as an immunomodulator in macrophages, causes an antiproteinuric effect in a mouse model of diabetes. Also, the treatment of cultured podocytes with GIT-27 has been found to ameliorate free fatty acid- or hyperglycemic-induced production of inflammatory cytokines in these cells. Another TLR4 antagonist, CRX-526, is observed to decrease streptozotocin (STZ)-induced inflammatory chemokines (CCL2 and CCL5) and subsequent macrophage infiltration and collagen deposition in endothelial nitric oxide synthase (eNOS) knockout mice kidney tissues, through dampening the activation of NF-κB and overexpression of TGF-β. Whether or not, the inhibition of other TLRs, including TLR3/5/7/8/11, exhibit renoprotective effect in cell or animal models of DKD, remains undetermined. Moreover, the perturbation of GM6135-miRNA203-TLR4 axis may attenuate the TLR4-mediated signaling, and thus, might serve as an alternative therapeutic approach to treat DKD. Despite having the convincing role of TLRs antagonists in treating DKD, future studies are needed to better understand their specificity and off-target effects, which eventually would be useful to resolve the issues of systemic immunosuppression and to conduct subsequent clinical trials.

#### 3.2 NLRs signaling inhibitors

Recent studies have presented several inhibitors of the NLRP3 inflammasome pathway that showed a curative effect in vitro and/or in vivo models of NLRP3-associated diseases, including DKD (Figure 2, panel B). Mechanistically, these inhibitors act by targeting either NLRP3 protein (NLRP3 inhibitors) or other components (ATPase, ASC, and caspase-1 inhibitors) of the NLRP3 inflammasome. MCC950, a potent, small-molecule inhibitor of both canonical and non-canonical activation of the NLRP3 inflammasome, inhibits the production of IL-β in both cultured mesangial cells and diabetic mice, which subsequently leads to reduced podocyte...
injury, renal fibrosis, and renal dysfunction.\textsuperscript{108} In addition, a caspase-1 inhibitor, M-920, is found to decrease albuminuria and the deposition of the renal extracellular matrix by impeding the activation of renal caspase-1, IL-1, IL-18, and NLRP3 inflammasome,\textsuperscript{109} thus, suggesting the importance of NLRP3 inflammasome inhibitors in treating DKD. Since the role of NLRC4 and NLRC5 is known in inducing DKD, identification and evaluating the effect of NLRP4 and NLRP5 selective inhibitors against DKD are needed in future studies. Specific targeting of NLRP3 or its components could be beneficial as it may overcome systemic immunosuppression without affecting nonspecific targets. Moreover, the impact of other NLRP3 inflammasome inhibitors that have shown therapeutic potential against other inflammatory diseases, could be tested for their effects in preclinical and clinical models of DKD.

### 3.3 Inflammatory mediators inhibitors

Considering the excessive activation of NF-κB and inflammatory mediators as a hallmark feature of DKD, reducing their levels is considered as a potentially efficacious therapy (Figure 2, panel A-D). The anti-NF-κB therapies are nonspecific, and they provide renal protection by indirectly inhibiting the NF-κB activity in mice.\textsuperscript{48,110} Among the DKD-associated inflammatory mediators, TNF-α is most extensively studied with respect to its inhibition and the subsequent effect on amelioration of DKD progression.\textsuperscript{54,111,112} So far, three types of anti-TNF-α therapeutic interventions have been evaluated: anti-TNF-α antibodies (infliximab), TNF-α inhibitor (SKF86002), and a repurposed small molecule (pentoxifylline). The treatment of rat with infliximab abolished TNF-α action, which led to reduced urinary excretion of albumin and TNF-α and ameliorated DKD in treated diabetic mice.\textsuperscript{113,114} The SKF86002 inhibitor significantly halted renal TNF-α levels and eventually, enhanced kidney function during glomerulonephritis.\textsuperscript{115} Pentoxifylline, originally as a hemorrhagic agent, is also known to have an anti-TNF-α transcriptional activity, and thus, has been employed as a suitable drug against several inflammatory diseases, including DKD.\textsuperscript{116} Additionally, pentoxifylline shows the ability to inhibit the activity of several other inflammatory mediators such as IL-1, IL-6, IFN-γ, VCAM1, and ICAM1.\textsuperscript{116}
several clinical studies of DKD, pentoxifylline has been found to reduce proteinuria in renal patients, suggesting it as a renoprotective agent to treat DKD. The inhibitors of other DKD-causing inflammatory mediators/receptors such as CCX140-B (CCR2 inhibitor) and NOX-E36 (CCL2 inhibitor) are currently under clinical trials, and thus, can be considered for the treatment of DKD. Alternatively, the use of CCX140-B in combination with the inhibitors of the renin-angiotensin-aldosterone system is assessed as renoprotective with reduced albuminuria in patients with diabetes. Hence, patients with albuminuria, without being interfered with the consistent blockage of the renin-angiotensin-aldosterone system, could experience from the beneficial anti-albuminuric effect of pentoxifylline, CCX140-B, or NOX-E36. Despite having a known protecting efficacy of anti-TNF-α drugs against DKD, further large-scale, adequately powered, and placebo-controlled trials with definitive efficacy and safety are needed to be conducted in the population with diabetes. The macrophage polarization-modulating therapies such as tectorigenin, pentraxin-3, and enalapril have also shown renoprotective effects in diabetic mice via curbing the production of inflammatory mediators; however, their effect remains to be evaluated in clinical trials.

The sodium-glucose cotransporter-2 (SGLT2) inhibitors have also emerged as new renoprotective agents that exert their activity in glucose lowering-dependent and -independent manners. The treatment of diabetic mice/rats with empagliflozin or ipragliflozin resulted in antihyperglycemic effects and diminished levels of CCL2, CCL5, IL-6, IL-β, TNF-α, NF-κB, and C-reactive protein in renal tissues or plasma samples. Dagapilfloxin improved hyperglycemia and reduced diabetes-mediated glomerulosclerosis by abrogating the expression of markers of inflammation (eg, CCL2, TGF-β, and NF-κB) and oxidative stress in renal tissues of diabetic mice. Canagliflozin provided renoprotective effects in diabetic mice by normalizing hyperglycemia as well as renal expression of CCL2 and TNF-α via inducing the AMP-activated kinase activation. In a post hoc analysis of a crossover clinical trial, dapagliflozin lowered the albuminuria and urinary excretion of IL-6 in treated individuals when compared to placebo. Moreover, canagliflozin decreased the amount of IL-6, matrix metalloproteinase (MMP) 7, TNF receptor 1, and fibronectin 1 in human plasma samples, suggesting its anti-inflammatory and anti-fibrotic activities to prevent the progression of DKD. Attention should be paid while using the SGLT2 inhibitors as they may confer side effects such as hypoglycemia, ketoacidosis, and urinary/genital tract infections.

The therapeutic impact of the inhibition of other inflammatory mediators such as ICAM-1 and VCAM-1, which are still at the stage of infancy, could be considered in the future for the development of novel therapeutic interventions against DKD.

### 3.4 Complement system inhibitors

Controlling the complement-mediated diseases has been a strong interest over the last five decades; however, despite such huge efforts, only a few drug candidates were able to exhibit promising therapeutic potential. For instance, neutralizing antibodies (OMS721, AMY-101, eculizumab, and IFX-1; target MASp2, C3, C5, and C5a, respectively), small molecules (CCX168, LNP023, and ACH4471; target C5aR1, factor B, and factor D, respectively), peptides (C1INH, APL2, and rVA576; target C1, C3, and C5, respectively), and oligonucleotides (Cemdisiran; targets C5) are complement inhibitors in clinical trials against glomerular diseases.

So far, only a few studies have evaluated the curative effect of blocking the complement signaling in DKD (Figure 2, panel C). Blockade of C3a and C5a receptors using receptor-specific antagonists prevented endothelial cells to myofibroblasts transition via inhibiting the downstream Wnt/β-catenin pathway, which resulted in alleviation of renal fibrosis in the mice model of diabetic kidney disease and in cultured human glomerular endothelial cells. Similarly, the use of a C3a receptor-specific blocker in a diabetic rat model also noticed to be renoprotective via dephosphorylating IκBα and inhibiting the TGF-β/SMAD3 signaling cascade. The specific targeting of the component C5a by the oligonucleotide NOX-D21 dampened the level of diacylglycerol acyltransferase-1 and sterol regulatory element-binding protein-1 and decreased glomerular and tubulointerstitial damage in diabetic mice kidney. Moreover, K-76COONa, another C5 inhibitor, ameliorated the severity of albuminuria and mesangial expansion in a mice model of diabetes.

The most appropriate approach to target the complement system to prevent the onset of DKD is currently unclear. Therefore, the field for the development of therapeutics targeting other DKD-causing complement components is widely open and demands the need for additional studies in the future. Also, the use of clinically proven anticomplement drugs against other diseases could be repurposed for DKD, which would be time-saving and may meet the demand of anti-DKD drugs in immediate future. The risks of long-term anticomplement therapy in chronic conditions should be carefully addressed as it may lead to immunosuppression and subsequently, make the condition more infection prone.

### 3.5 JAK-STAT signaling inhibitors

Since JAKs and STATs participate in a variety of diseases, several efforts have been made in developing the specific inhibitors of the JAK-STAT pathway. Currently, there are two JAK-specific inhibitors that have been approved by the FDA: ruxolitinib (JAK1 and JAK2 inhibitor) and tofacitinib (JAK1 and JAK3 inhibitor). Ruxolitinib is approved for the treatment...
of polycythemia vera and myelofibrosis, whereas tofacitinib is approved as an immunosuppressive agent for the treatment of autoimmune diseases. Other inhibitors, including baricitinib (JAK1 and JAK2 inhibitor) and VX-509 (JAK3 inhibitor), have also shown promising results in treating arthritis; and are supposed to be used clinically in the next few years. Among these inhibitors, the therapeutic effect of baricitinib against DKD has been tested in the phase-II clinical trial (Figure 2, panel D). When compared with the placebo, the baricitinib treatment of patients suffering from DKD-reduced albuminuria. The use of ruxolitinib, tofacitinib, and VX-509 against DKD is likely to be effective, but their effects are needed to be evaluated in future studies. Several natural and synthetic inhibitors of STAT proteins have also been developed; however, only a limited number of clinical studies are currently in progress. As the JAKs and STATs antagonists are immunosuppressive in nature, short-term therapy with a high dose or long-term therapy with a low dose can be prescribed to prevent infection-related complications.

4 CONCLUDING REMARKS

DKD results in high morbidity and mortality in the human population. Despite the presence of effective antidiabetic drugs, the incidence of DKD is increasing. Research progress made in recent years have advanced our understanding of the basic mechanisms involved in DKD, and have pointed out new therapeutic targets to overcome DKD. Several therapeutic options that have shown promising results in preclinical mice studies could be employed for clinical trials without any delay. Considering the importance of inflammatory pathways in regulating DKD, a combination treatment strategy including both anti-inflammatory and antidiabetic drugs might provide better protection against DKD. Since innate immune receptors and molecules induce antimicrobial and antitumor activities, their inhibition may promote immune suppression and malignancies in patients with diabetes. Therefore, the long-term usage of anti-inflammatory drugs should be advised with caution, and these drugs are needed to be further evaluated in future studies.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
SF Wan, SK Wan, XJ Jiao, and HX Cao searched literature and designed manuscript. SF Wan, SK Wan, XJ Jiao, HX Cao, and Y. Gu wrote manuscript. SF Wan and Y. Gu prepared figures. L. Yan, Y. Zheng, PY Niu, and FM Shao reviewed manuscript, edited, and gave critics. All authors approved the final version of the manuscript.

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