


The Role of Immune Cells in DKD: Mechanisms and Targeted Therapies

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Abstract: Diabetic kidney disease (DKD), is a common microvascular complication and a major cause of death in patients with diabetes. Disorders of immune cells and immune cytokines can accelerate DKD development of in a number of ways. As the kidney is composed of complex and highly differentiated cells, the interactions among different cell types and immune cells play important regulatory roles in disease development. Here, we summarize the latest research into the molecular mechanisms underlying the interactions among various immune and renal cells in DKD. In addition, we discuss the most recent studies related to single cell technology and bioinformatics analysis in the field of DKD. The aims of our review were to explore immune cells as potential therapeutic targets in DKD and provide some guidance for future clinical treatments.

Keywords: diabetic kidney disease, immune cells, bioinformatics analysis, single-cell analysis

Introduction

The prevalence of diabetes in people aged between 20 and 79 years worldwide was forecast to be 10.5% in 2021 and is expected to rise to 12.2% by 2045.¹⁻⁴ Diabetic kidney disease (DKD), a chronic kidney disease resulting from diabetes, is defined by persistent proteinuria and ongoing deterioration in renal function, and affects 20% to 50% of patients with diabetes.⁵ DKD is the main cause of end-stage renal disease (ESRD) and among the most common microvascular complications of diabetes.⁶⁻⁹ Glomerular hypertrophy, tubulointerstitial inflammation, fibrosis, and glomerulosclerosis are pathological conditions caused by metabolic alterations associated with diabetes.¹⁰⁻¹⁵

DKD has a complicated and multifaceted pathogenesis, involving a combination of oxidative stress, the renin-angiotensin-aldosterone system (RAAS), the immune system, and inflammation.¹⁶ The conventional view is that DKD is a non-immune, metabolic, or hemodynamic glomerular disease caused by hyperglycemia, however research, has discovered that proinflammatory cytokine release and low-grade inflammation, both systemic and local, which are mainly due to inflammation driven by the innate immune system, are linked to DKD onset and progression.¹⁷ Abnormal metabolic states such as hyperglycemia and hyperinsulinemia lead to damage to the glomerular filtration membrane and apoptosis of renal tubular epithelial cells (TECs), and these passive effects elicit a response from the immune system.¹⁸⁻²¹ Immune cells recognize damage to kidney cells and release various cytokines to further promote the inflammatory response, driving kidney structure remodeling and interstitial fibrosis.^{17,22} Macrophages are a major class of innate immune cells, while T cells are important for adaptive immunity. Numerous experimental models of DKD and clinical trials have revealed the infiltration and activation of T lymphocytes and macrophages in the kidney.²³⁻²⁷

The number of individuals with ESRD resulting from DKD is rising annually, despite notable therapeutic advances,²⁸ these patients require dialysis and kidney transplantation. The immune system contributes significantly to DKD; however, more research is needed to determine the exact mechanism by which immunity mediates the condition.

Role of the Immune System in DKD

The immune system comprises innate and adaptive arms. There are various classes of pattern recognition receptors (PRRs) in the innate immune system, such as nucleotide-binding NOD-like receptors (NLRs) and membrane-bound Toll-like receptors (TLRs), which recognize pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) in both extracellular and intracellular contexts.¹⁷ Unlike the adaptive immune system, the innate immune system recognizes endogenous danger signals by recognizing DAMPs, in addition to microbial byproducts, and can identify and eradicate the majority of microorganism-caused damage in a matter of minutes or hours.²⁹ Rapid-acting cells of the innate immune response comprise granulocytes, mast cells, macrophages, and dendritic cells (DCs). Conversely, specificity, diversity, and memory are critical characteristics of the adaptive immune response, which differentiates self from non-self through specialized defense mechanisms involving specific recognition molecules.³⁰ The primary cellular components of the adaptive immune system are T and B cells.

The innate immune system has a vital role in DKD pathogenesis. Prolonged exposure to high levels of blood glucose and advanced glycation end products (AGEs) results in injury or death of renal cells, leading to the release of DAMPs into the extracellular space. This danger signal is recognized by PRRs like TLRs (TLR2 and TLR4), NLRs, protease-activated receptors in the kinin-kallikrein system, the complement cascade, and other cell surface receptors. Activating these receptors triggers an inflammatory response in kidney-associated cells, while kidney injury leads to entry of monocytes from the bone marrow into the circulation, which differentiate monocytes into inflammatory macrophages and an increase in adhesion molecule expression on vascular endothelial cells. Further, the process of macrophage infiltration into damaged kidneys amplifies the inflammatory response. As signaling pathways, including transforming growth factor- β (TGF- β), mitogen-activated protein kinase (MAPK), and nuclear factor kappa-B (NF- κ B), are activated, chronic and unresolved nephritis develops into progressive renal fibrosis³¹ (Figure 1). The complement system is crucial in innate immune defense via interactions with PRRs and drives inflammation. Glycosylation-induced inactivation of CD59 and hyperglycemia-induced activation of complement signaling combine to increase membrane attack complex deposition in tissues, which, in turn, activates intracellular signaling pathways leading to the release of proinflammatory cytokines and growth factors.³² There is growing evidence that the complement system contributes to DKD development. Additionally, complement activation can be used to identify patients at risk of this complication and serves as a target for therapy.³³

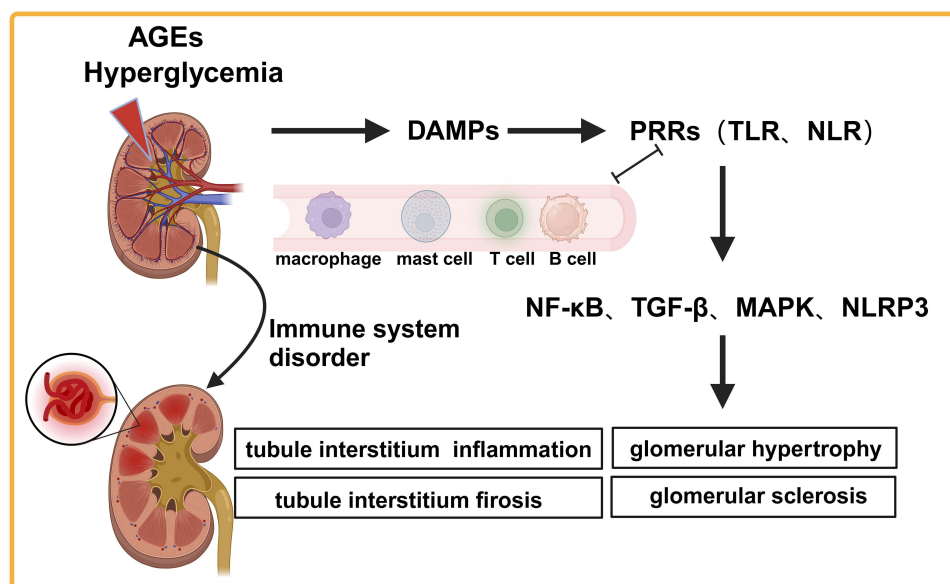


Figure 1 Immune cells are involved in DKD development.

Abbreviations: AGEs, advanced glycation end products; DAMPs, danger-associated molecular patterns; PRRs, pattern recognition receptors; TLR, Toll-like receptors; NLR, NOD-like receptors; NF- κ B, nuclear factor kappa-B; TGF- β , transforming growth factor- β ; MAPK, mitogen-activated protein kinase; NLRP3, nucleotide-binding domain, leucine-rich repeat, and pyrin domain-containing protein 3.

The adaptive immune system mainly functions through T and B cell activity. The kidneys of patients with type 2 diabetes mellitus (T2DM) have elevated activated T cell infiltration relative to those of nondiabetic patients, which is correlated with the degree of proteinuria in these patients.³⁴ Further, increases in the percentage of Th17 cells and decreases in regulatory T cell (Treg) levels have been discovered in the blood of patients with type 1 diabetes; this disruption of the Treg/Th17 balance may exacerbate the development of microvascular complications of diabetes.³⁵ Further, there is evidence implying that synergistic interaction of the adaptive immune system with specific inflammatory cytokines may be a fundamental element in the development of diabetic kidney damage.²³ Additional research into the interactions between T cells and other immune cells in DKD, as well as examination of the differentiation and function of different T cell subpopulations in the context of dysregulated immune homeostasis, is necessary. Such research will be crucial for improving disease prediction and treatment.

Role of Immune Cells in DKD

Macrophage

Macrophages are major immune cells that infiltrate the kidneys in DKD, and their recruitment and activation promote the development of sclerosis and renal injury in individuals with diabetes.³⁶ Further, activation of infiltrating and resident macrophages in the diabetic kidney leads to renal fibrosis and inflammation in the glomerular and tubulointerstitial compartments.³⁷ Resident macrophages, which act as gatekeepers in the activation or inhibition of immunological responses, are rapidly activated by stimulation, and contribute significantly to DKD by producing cytokines and chemokines, attracting peripheral monocytes and macrophages, intensifying renal-associated cellular damage, and transforming into macrophage-myofibroblasts.³⁸

Macrophages can be broadly categorized into M1 and M2 types according to their function and activation state. In the initial phases of renal damage, proinflammatory cytokines, DAMPs, PAMPs, and interferon gamma (IFN- γ) activate macrophages, initiating their transformation into a cells with an M1 phenotype, which are proinflammatory and react to cell damage.³⁹ M1 macrophages contribute to inflammation by producing inducible nitric oxide synthase, as well as enhancing tissue inflammation and injury via the secretion of proinflammatory cytokines. Conversely, M2 macrophages reduce inflammation and promote fibrosis and wound repair.⁴⁰ The M1/M2 macrophage ratio varies dynamically as DKD progresses through different phases. Renal biopsies revealed that, in the early stages of DKD (stages I and IIa), M1 macrophages are recruited to the kidney. The M1/M2 macrophage ratio peaks in the early stage of DKD and drops to a minimum later in the disease (stage III), when M2 macrophages become dominant.⁴¹ In renal samples from patients with DKD, glomerular CD163⁺ (an M2/anti-inflammatory marker) macrophages are positively correlated with DKD grade, interstitial fibrosis, tubular atrophy, and glomerulosclerosis.³⁶

The regulation of macrophage recruitment and activation in DKD is a complex process involving various mechanisms. High glucose levels promote M1 macrophage transformation and podocyte apoptosis while reducing Sirt6 expression. Conversely, the overexpression of Sirt6 enables podocyte protection through M2 macrophage activation.⁴² Factors such as hyperglycemia, oxidative stress, and DAMPs promote the activation of the nucleotide-binding domain, leucine-rich repeat, and pyrin domain-containing protein 3 (NLRP3) inflammasome in macrophages, leading to the production and release of inflammatory cytokines, such as Interleukin-1 Beta (IL-1 β) and Interleukin-18 (IL-18), which exacerbate the inflammatory response. NLRP3 knockout (KO) mice exhibit improved renal function, reduced glomerular hypertrophy, glomerulosclerosis, and inflammation under diabetic conditions.⁴³ CAY10603, as a specific inhibitor of HDAC6 (histone deacetylase 6), significantly reduces pyroptosis in macrophages by suppressing the activation of the NLRP3 inflammasome, thereby demonstrating its therapeutic potential in the treatment of DKD.⁴⁴ Bruton's Tyrosine Kinase (BTK), an essential factor in immunomodulation, is activated in the kidneys of patients with DKD, and this activation correlates with proteinuria, creatinine levels, estimated glomerular filtration rate (eGFR), and progression of renal pathological changes.⁴⁵ BTK KO in a mouse model has been shown to alleviate diabetic kidney damage, characterized by reduced urinary protein and decreased kidney inflammation, which is associated with reduced activation of the NLRP3 inflammasome in macrophages.⁴⁵ A BTK inhibitor (PCI-32765) blocks macrophage-generated MAPK and NF- κ B pathways, countering the pro-inflammatory consequences of high glucose,⁴⁶ suggesting that it has potential as an

immunosuppressant against DKD-induced inflammation, particularly hyperglycemia-induced responses. Variants of the gene encoding tonicity-responsive enhancer-binding protein (TonEBP) are robustly associated with inflammation and renal function in healthy populations. Furthermore, TonEBP mediates pro-inflammatory activation and macrophage migration induced by hyperglycemia in a DKD mouse model, leading to increased inflammation and renal injury.⁴⁷

There is significant deposition of ApoC1 in the glomeruli of kidneys from patients with DKD, and ApoC1 over-expression can increase TNF- α and TGF- β cytokine-induced glomerulosclerosis in macrophages.⁴⁸ Glycosphingolipid expression is also increased in DKD kidneys, which mediates inflammatory responses by enhancing TLR4 in human monocytes and mouse bone marrow-derived macrophages.⁴⁹ Serum mannose-binding lectin (MBL) is a complement-activated molecule that recognizes carbohydrates and is associated with DKD. MBL triggers inflammation in macrophages by activating the NF- κ B pathway and promotes macrophage conversion to the M1 phenotype, independently of the complement lectin pathway.⁵⁰ P2X7R is among multiple adenosine-5'-triphosphate-activated receptors; is expressed in macrophages, mesangial cells, and other cell types; and regulates MCP-1 secretion in cell models. Renal biopsy samples from patients with DKD exhibit high P2X7R expression levels, and mesangial P2X7R activation can stimulate early glomerular macrophage recruitment and prioritize activation of M1-polarized pro-inflammatory macrophages. A selective P2X7R inhibitor has potential to reduce renal macrophage accumulation, provide renal protection, and become a therapeutic agent in the future.⁵¹

Immune cells, particularly macrophages, and intrinsic renal cells interact closely in DKD to promote disease progression (Figure 2). In diabetes, the conditions of high glucose, AGEs, and albumin levels lead to increased heparinase expression in glomerular cells, where heparinase stimulates macrophage-mediated inflammation, leading to chronic kidney injury.⁵² Hence, heparinase is associated with macrophage activation, chronic inflammation, and kidney injury in diabetes. In DKD kidneys, IL-1 β is mainly derived from renal TECs, which promote macrophage polarization towards a pro-inflammatory M1 phenotype and secretion of IL-6, leading to sodium retention and salt sensitivity.¹⁸ M1

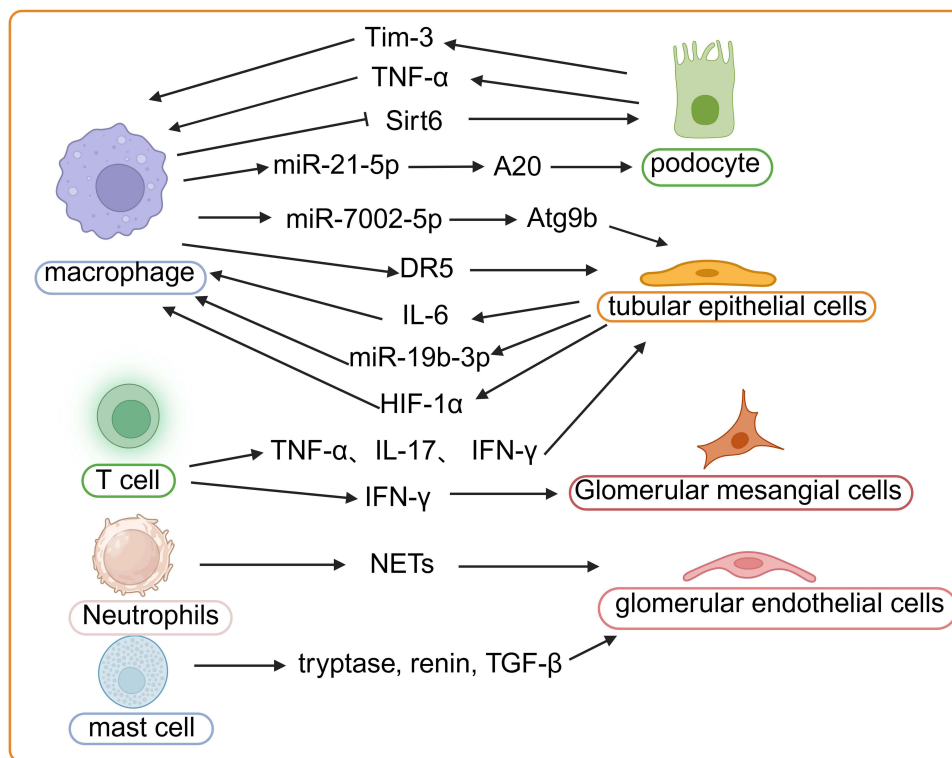


Figure 2 Interactions of immune and renal cells.

Abbreviations: Tim-3, T-cell immunoglobulin structural domain and mucin structural domain-3; TNF- α , tumor necrosis factor-alpha; Sirt6, sirtuin 6; Atg9b, autophagy-related protein 9B; A20, tumor necrosis factor alpha-induced protein 3; DR5, death receptor 5; IL-6, interleukin-6; HIF-1 α , hypoxia-inducible factor 1-alpha; IL-17, interleukin-17; IFN- γ , interferon gamma; NETs, neutrophil extracellular traps; TGF- β , transforming growth factor- β .

macrophages accelerate cellular senescence by inducing elevated reactive oxygen species levels and p38 MAPK signaling activation in human renal glomerular endothelial cells.⁵³ Plasma growth hormone (GH) levels are increased in individuals with type 1 diabetes, and GH-stimulated podocytes have the potential to cause macrophage accumulation by secreting TNF- α and prompting macrophage migration, potentially serving as an independent factor mediating DKD progression in patients with type 1 diabetes.⁵⁴ In diabetic mice, a reduction in angiotensin II type 1 receptor-associated protein within the renal tubules results in hyperactivation of the tubular angiotensin II type 1 receptor signaling/RAAS system, while CD206 expression, which is considered a marker of M2 macrophages, is decreased in the renal tubular interstitium. Tubulo-glomerular crosstalk leads to increased levels of TNF- α and oxidative stress components in the glomerulus, as well as exacerbation of tubular hypertrophy and podocyte injury.⁵⁵ T-cell immunoglobulin structural domain and mucin structural domain-3 (Tim-3) are a negative regulator of Th1 immunity and have complex roles in regulating macrophage activation. Increased Tim-3 expression in renal macrophages through activation of the NF- κ B/TNF- α pathway was found in patients with DKD and diabetic mice, as well as damaged podocytes.⁵⁶ The knockout of angiotensin-like protein 3 alleviates podocyte epithelial–mesenchymal transition and injury in DKD by promoting the transformation of M1 to M2 macrophages, inhibiting NLRP3 inflammasome activation, and suppressing IL-1 β release.⁵⁷

Non-coding RNAs and exosomes contribute to the interactions between kidney cells and macrophages in DKD. Elevated concentrations of miR-19b-3p in urine exosomes are correlated with tubulointerstitial inflammation in patients with DKD. Furthermore, damaged renal TECs communicate with macrophages through the exosome/miR-19b-3p/SOCS1 axis, leading to M1 macrophages activation and exacerbation of tubulointerstitial inflammation.⁵⁸ Macrophage infiltration around lipotoxic renal TECs has also been detected in patients with DKD, and lipotoxic TEC-derived extracellular vesicles (EV) activate the inflammatory phenotype of macrophages and induce production of macrophage-derived EV, which can induce TEC apoptosis by a death receptor 5 (DR5)-dependent process.¹⁹ Furthermore, miR-21-5p in macrophage-derived EV can regulate A20 and cause podocyte injury.⁵⁹ High levels of proteinuria can stimulate the expression of inflammatory and fibrotic genes in DKD, by increasing HIF-1 α -induced glycolysis in renal macrophages, through enhancement of EV production by renal TECs.⁶⁰ Macrophage-derived exosomes stimulated by high glucose (HG-exo) cause renal mesangial proliferation and extracellular matrix (ECM) accumulation. HG-exo induces dysfunction and inflammation in mouse TECs by carrying excess miR-7002-5p and inhibits autophagy by targeting Atg9b.⁶¹ Glomerular mesangial cells (GMCs) are involved in DKD development by promoting NLRP3 inflammasome activation and autophagy defects in response to high glucose-treated macrophage-derived exosomes.⁶² Furthermore, Epsin1 mediates the activation of exosome sorting induced by Dll4 and Notch1, to regulate tubular macrophage crosstalk in DKD.⁶³ In contrast, miR-93-5p and TLR4 expression are upregulated by exosomes released by M2 macrophages, which reduces LPS-induced podocyte apoptosis.⁶⁴ M2 macrophages also ameliorate high-glucose-mediated podocyte injury by suppressing DUSP1 expression, secreting exosomes containing miR-25-3p, and activating cellular autophagy.⁶⁵

T Cells

T lymphocytes are classified into CD4 and CD8 subpopulations, based on cell surface markers, and have vital functions in pathogen removal and host defense. Based on their cytokine profiles and functional roles, CD4⁺ T cells can be further subdivided into T helper (Th) cells and Tregs. Antigens in the context of class I major histocompatibility (MHC-I) molecules are specifically recognized by CD8⁺ T cells, which mainly function as cytotoxic T cells. CD8⁺ and CD4⁺ T cells are defined as conventional T cells; however, unconventional T cells, that respond rapidly upon encountering antigen, are also present in the kidney, including mucosa-associated invariant T, natural killer T and $\gamma\delta$ T cells.⁶⁶ Additionally, a class of T cells known to reside in the kidney has been identified as tissue-resident memory (TRM) T cells, which are the most prevalent subpopulation of memory T cells.⁶⁷ These different T cell subtypes regulate one another in various ways and work together to maintain immune homeostasis in the kidney.

T cells, and their activation markers, are actively expressed in the periphery and kidney in DKD. Kidneys from diabetic mice have significantly increased levels of CD3⁺ T cells, which generate TNF- α and IFN- γ . BTBR ob/ob mice have a phenotype similar to advanced human DKD, with increased infiltration of T lymphocytes (CD3⁺, CD4⁺, and $\gamma\delta$ lymphocytes) and increased expression of Th17 drivers, such as IL-6, observed in their kidneys.²⁸ Furthermore, patients with type 2 diabetes have significantly higher levels of CD4⁺, CD8⁺, and CD20⁺ cells in the renal interstitium, and their

proteinuria levels are correlated with CD4⁺ and CD20⁺ cell numbers.³⁴ Furthermore, the numbers of IFN- γ ⁺CD4⁺ and IL-17A⁺CD4⁺ T cells in the kidneys increase significantly from the onset of albuminuria, and the common immunosuppressant, mycophenolate mofetil, can suppress intrarenal IL-17A⁺ CD4⁺ T cells from early DKD and ameliorate proteinuria.²⁷ KIM-1 and CD8⁺ T cell infiltrates were detected in renal biopsies from patients with DKD. Glycemic changes increase KIM-1 production from CD8⁺ T cells, thus raising the risk of DKD in patients with diabetes.⁶⁸ Increased numbers of peripheral blood CD4⁺CR5⁺PD-1⁺ THF cells were also found in patients with DKD,⁶⁹ and patients with type 2 diabetes and proteinuria express more CD25 and CD69 on their circulating CD8⁺ T cells.⁷⁰ Moreover, the serum levels of T-lymphocyte activation markers, specifically CD28 and CTLA-4, increase as DKD progresses. Low eGFR levels and high urinary TNF- α are positively correlated with CTLA-4, indicating that activation of the TNF- α pathway and T-lymphocyte-mediated immune responses are important in the early stages of DKD.⁷¹ In patients with DKD, there is a relationship between renal CD3⁺ T cell accumulation and urinary CCL21 levels, which are derived from small EV and co-localize with CCL21 in the tubular interstitium. CCL21-mediated T cell infiltration may be an important chronic inflammation pathway in DKD.⁷² Analysis of peripheral blood samples from patients, with early DKD using the mass cell counting technique, CyTOF, revealed a significant decrease in B cells, a significant increase in monocytes, and a notable increase in Th1 cells and Treg.⁷³

Several studies have reported a correlation between elevations of T cell counts and levels of their activation markers with DKD and indicators, such as renal function, suggesting that T cell activation is a significant factor in DKD. Studies on renal fibrosis have demonstrated that T cells stimulate renal TECs to produce specific chemokines, such as CCL2 and CXCL10, through the release of pro-inflammatory factors, including TNF- α and IL-17, thus recruiting more T cells and other immune effectors to the kidney and inducing renal TECs to transform into mesenchymal fibroblast-like cells. Further, by releasing fibroblastogenic factors, such as TGF- β , immune effectors activate mesenchymal fibroblasts and promote their proliferation and secretion of stromal matrix.⁷⁴ Among T cells, Th cells primarily promote or inhibit inflammatory responses by secreting cytokines, including TNF- α , IFN- γ , IL-17, IL-10, and TGF- β , which impact renal inflammation, repair, and fibrosis.⁷⁵ It is currently thought that, in DKD, T cells are either expanded, differentiated, and activated in the kidney, or are recruited from the circulation to renal tissues. These cells can have either harmful or beneficial effects through various mechanisms, including modifying insulin resistance, injuring podocytes, encouraging fibrosis, and controlling proteinuria. Th1, Th2, Th17, Treg, and cytotoxic T cells have all been implicated in the development of DKD.⁷⁶

Investigation of the roles of T cells in DKD has become the focus of significant attention, and some potential mechanisms by which T cells promote DKD and targets for T cell regulation have recently been discovered. Biglycan is an ECM proteoglycan that is upregulated at all stages of DKD, and stimulates CXCL10 and CCL20 production by macrophages through the biglycan/TLR/TRIF/myd88 signaling pathway, thereby recruiting Th1 and Th17 cells. Further, IFN- γ can synergistically stimulate macrophages to enhance CXCL9 expression and attract CXCR⁺ Th1 and Th17 cells, enhancing the infiltration of T cell subsets.⁷⁷ In addition, IFN- γ can induce GMCs to express antigen-presenting and co-stimulatory molecules, such as MHC-II, ICAM-1, CD40, and CD80, which act as non-professional antigen-presenting cells, while uptake and presentation of antigenic peptides by activated GMCs induces CD4⁺ T cell (Th0 cells) polarization toward a Th1 phenotype and promotes inflammatory responses.⁷⁸ Liang Li et al⁷⁹ found that the percentage of CD8⁺ TRM cells was significantly increased in kidneys from DKD mice and patients. IL-15, which is highly expressed in DKD kidneys, significantly promotes the development and activation of CD8⁺ TRM cells, thereby inducing podocyte injury and glomerulosclerosis. Sparsentan can regulate renal CD8⁺ TRM cells by blocking angiotensin II endothelin-1 mediated IL-15 signaling. In kidney biopsy specimens from patients with type 2 diabetes, the number of CD4⁺IL-17⁺ T cells is positively associated with eGFR deterioration, and IL-6 may be synergistically enhanced by IL-17 and CD40L. Further, production of MCP-1, TGF- β , and NF- κ B mediates the inflammatory response and remodeling linked with tissue damage and glomerulosclerosis in DKD.⁸⁰

Mast Cells

Mast cells are pluripotent cells derived from bone marrow that contain abundant mediators of inflammation and growth factors. Mast cell precursors are hematopoietic progenitor cells that migrate to injured tissue from the bone marrow and

mature into local mast cells. Resident renal mast cells may contribute to DKD development by releasing various pathogenic substances, including chymase, TGF- β , cathepsin G, tryptase, renin, histamine, and proinflammatory cytokines.⁸¹ Further research is required to validate the potential role of these mast cell-derived mediators in the intricate pathophysiology of DKD.

Immunohistochemical staining of renal tissues from patients with DKD of varying clinical stages revealed increases in the degree of degranulation and quantity of mast cells as the disease progressed. Mast cells may induce renal inflammation and fibrosis by degranulating and releasing bioactive chemicals into the tubular interstitium, including chymase, tryptase, TGF- β 1, renin, and TNF- α .⁸² Rats with DKD kidneys also showed an increase in mast cell counts, while application of the renin-angiotensin system blocker, aliskiren (AL), mitigated the rise in mast cell numbers, indicating a potential protective effect of AL on the kidney,⁸³ however, the exact mechanism involved requires further investigation. Mast cell infiltration may cause renal interstitial fibrosis through the SCF/c-kit signaling pathway in a rat model of DKD, while tranilast, an anti-allergic medication, can prevent renal interstitial fibrosis by reducing mast cell infiltration in this context.⁸⁴ SERPINA3, an inhibitor of mast cell chymase, is upregulated in the renal tubules of patients with DKD, and may serve as a protective immune-associated molecule that can prevent renal interstitial fibrosis by inhibiting mast cell activation and multiplication, as well as downregulating chymotrypsin activity, to prevent renal tubular injury.⁸⁵

B Cells

B lymphocytes are derived from pluripotent stem cells in the bone marrow, which transform into plasma cells in response to antigenic stimulation. Plasma cells produce and release antibodies, thereby contributing to humoral immunity.⁸⁶

B-cell infiltration and immune complex deposition have been observed in kidneys affected by DKD. A study conducted on children with type 1 diabetes mellitus demonstrated a relationship between elevated levels of circulating IgG immune complex and the development of early DKD.⁸⁷ In addition, increased deposition of IgG+ B cells was detected in the glomeruli of diabetic NOD mice.⁸⁸ Furthermore, immune complexes containing oxidized low-density lipoprotein cholesterol are thought to promote mesangial expansion in the glomeruli by stimulating type IV collagen production.⁸⁹ Among B cell subsets, CD38⁺CD19⁺ and CD38⁺CD19⁺CD40⁺ B cells were significantly elevated in peripheral blood from both patients with DKD and healthy controls, while CD38⁺CD19⁺ B cells number was positively correlated with serum IgG levels and albumin excretion rate and negatively correlated with eGFR.⁶⁹ Consequently, B cells are thought to contribute to DKD by generating antibodies and building immune complexes that are deposited in the kidney.

Immunological complexes in the glomerulus trigger complement activation and cytokine release, which in turn drive macrophage aggregation and inflammation. The release of DAMPs after ECM injury in DKD may activate B cells.⁹⁰ Regulatory B cells (Bregs), a unique B cell subpopulation, downregulate immune responses, and their absence is linked to increased autoimmune responses. Bregs cells secrete IL-10 to maintain immune tolerance in the body. Numbers of circulating CD19⁺CD24^{hi}CD38^{hi} Bregs are reduced in patients with DKD. Further, the number of circulating CD19⁺CD24^{hi}CD38^{hi} B cells in patients with DKD is positively correlated with eGFR and serum IL-10 levels, but negatively correlated with urinary protein levels,⁹¹ implying that DKD development also involves Bregs. The potential beneficial effects of Bregs represent a novel avenue for exploration in the context of DKD treatment; however, studies on B cells in DKD are scarce and further exploration is necessary.

Neutrophils

Neutrophils are a subtype of granulocytes and the most abundant type of white blood cells with important functions in the immune system. These phagocytes have short life spans and are quickly mobilized to sites of infection or tissue injury, however, their involvement in DKD remains unclear. The neutrophil-lymphocyte ratio (NLR) is higher in patients with diabetes and nephropathy, and NLR is positively correlated with C-reactive protein levels and microalbuminuria, but negatively correlated with eGFR.^{92,93} The NLR is also used to evaluate the degree of neutrophil-related chronic inflammation (NRCI); however, a randomized, double-blind, controlled clinical trial discovered that, while low-dose colchicine therapy (0.5 mg/day) was effective and safe in reducing NRCI, it did not delay the onset of DKD.⁹⁴

Activation of neutrophils causes DNA decondensation and histone deamination, particularly of peptidylarginine deiminase 4 (PAD4), which catalyzes histone citrullination.⁹⁵ Subsequently, neutrophil extracellular traps (NETs),

which are composed of histones, DNA, and neutrophil proteases, such as neutrophil elastase (NE) and myeloperoxidase, are released.⁹⁶ Increased deposition of NETs has been identified in the glomeruli of patients and mice with DKD.^{97,98} NETs induce dysregulation of several genes related to cellular membrane function, resulting in pore formation in cell membranes and pyroptosis in glomerular endothelial cells.⁹⁷ Under high glucose conditions, NETs can enhance glomerular endothelial dysfunction and NLRP3 inflammasome activation both in vivo and in vitro, whereas inhibition of NETs (eg, with PAD4 inhibitors) can alleviate endothelial dysfunction and renal damage in DKD.⁹⁸ In T2DM, inflammation and neutrophil activation promote NE release, which is mediated by regulation of the endogenous inhibitors, alpha₁-antitrypsin (α_1 -AT) and alpha₂-macroglobulin (α_2 -MG), under normal physiological conditions. NE activity levels are significantly elevated in the plasma of patients with DKD, while α_1 -AT and α_2 -MG levels are decreased.⁹⁹ In summary, therapy targeting reduction of NETs secretion and maintenance of NE homeostasis may be promising for halting DKD progression.

Group 2 Innate Lymphoid Cells (ILC2s)

ILC2s are innate immune cells that respond rapidly to their surroundings via soluble inflammatory mediators and intercellular interaction¹⁰⁰ and play a critical role in Th1/Th2 homeostasis.¹⁰¹ ILC2s contribute to tissue healing, allergic inflammation, metabolic balance, and tissue fibrosis, including pulmonary fibrosis, intestinal fibrosis, and hepatic fibrosis.^{102,103}

Although less studied, ILC2s may also impact the course of DKD. In patients with DKD, mRNA levels of factors encoding the ILC2 associated molecules, ROR, T1/ST2, and IL-5/IL-13, are increased. Further, IL-13 and T1/ST2 mRNA expression levels are associated with fasting glucose, lipids, and body weight,¹⁰⁴ indicating that ILC2s may contribute to DKD by regulating blood pressure, lipid metabolism, and other factors that contribute to metabolic syndrome. Cuiping Liu et al¹⁰⁵ discovered that the proportions of ILC2 and Th2-associated cytokines, IL-4, IL-5 and IL-13, are higher in peripheral blood from patients with DKD, and positively associated with DKD severity. Furthermore, when HK-2 cells were stimulated with IL-4 or IL-13, TGF-1 and fibronectin (FN) production increased, indicating that ILC2s may have a role in renal fibrosis in DKD via the TGF-1 signaling pathway.

Single-Cell Analysis of Immune Cells in DKD

The development of single-cell RNA sequencing (scRNA-seq) techniques in recent years has increased the recognition of the roles of immune cells in renal disease.¹⁰⁶ Unlike bulk RNA sequencing, scRNA-seq can identify transcription status and signaling pathways in many cell types while quantifying gene expression in a single cell. A research project using both scRNA-seq and bulk RNA-seq revealed that the recruitment and activation of M1 macrophages and cytotoxic T cells initiates inflammation, which is a major contributor to permanent kidney damage.¹⁰⁷ Recent developments in library preparation and separation techniques, such as single nucleus RNA sequencing (snRNA-seq), have facilitated discovery of unusual cell types in cryopreserved samples.¹⁰⁸ Relative to the control group, snRNA-seq of renal biopsy specimens from individuals with type 2 diabetes and early-stage DKD showed a 7- to 8-fold increase in leukocytes. Additionally, increased expression levels of TNFRSF21 in infiltrating CD14⁺ monocyte subpopulations, IL-1R1 in CD16⁺ monocytes and antigen-presenting cells, and IL-18R1 in CD4⁺ and CD8⁺ T cells were detected.¹⁰⁹ ScRNA-seq of glomeruli in diabetic mice identified a higher number of immune cells, where macrophages were predominant, with more cells expressing M1 than M2 phenotypic markers.¹¹⁰ Fu et al¹¹¹ conducted scRNA-seq on CD45-enriched kidney immune cells from DKD mice and focused on analysis of mononuclear phagocytes. They found that, as DKD progressed, resident and infiltrating macrophage subpopulations in the kidneys increased, accompanied by subpopulation-specific increases in pro- or anti-inflammatory gene expression and gene expression tending towards an undifferentiated phenotype, while M1-like inflammatory phenotypes increased over time. Furthermore, based on scRNA-seq data from db/db mice kidneys, it has been predicted that M1 macrophages can target renal stromal cells via SPP1 and activate T cells via the MHC-II pathway, which then recognize other renal cells via the MHC-I pathway,¹¹² however, this study by Fu et al raises several unanswered questions.¹¹³ Renal inflammation is a result of intricate cell-cell communication among various cell types. The interaction between the identified subpopulation of activated macrophages and the renal parenchyma remains unclear, due to loss of spatial information during cell dissociation. The evolving field of transcriptomics, which has spatial resolution capabilities, may provide answers to these questions. Second, the regulation of

macrophage activation and polarization at the genomic level in DKD remains largely unexplored. Understanding of gene regulatory networks necessitates thorough analysis of multi-group single-cell datasets comprising transcriptomes and chromatin accessibility within the same cell. Furthermore, a comprehensive investigation into immune cell heterogeneity in human DKD is imperative, to augment findings garnered from preclinical mouse models.

Regulation of gene expression involves a dynamic interplay between control of chromatin structure and accessibility, and recruitment of transcription factors to promoter regions, enhancers, and activator sequences.¹¹⁴ Accessible chromatin regions can be identified using DNase-seq or assay for transposase accessible chromatin with high-throughput sequencing (ATAC-seq).¹¹⁵ Single-cell ATAC-seq studies of epigenomic landscapes promise to reveal heterogeneity among cells in gene regulatory programs;¹¹⁶ nevertheless, there has been a lack of ATAC-seq studies in the context of DKD.

ScRNA-seq and ATAC-seq datasets are generated post-tissue isolation, leading to loss of spatial and morphological details about cells in the tissue environment. This knowledge is vital to understand the role of various cells in tissue function, particularly for organs with intricate spatial structures, such as the kidney. The progressive development of spatial transcriptomics may address these issues, but no high-throughput spatial transcriptomics studies on the kidney have yet been published. In the future, new insights into renal homeostasis and disease are expected to be provided by spatial transcriptomics.

Additionally, the field of single-cell proteomics is developing rapidly. Single-cell proteomics technologies, including CITE-Seq (Cellular Indexing of Transcriptomes and Epitopes by Sequencing), have recently emerged, and can be combined with sequencing-based methods to simultaneously measure proteins and transcripts at single-cell resolution.¹¹⁷ It is anticipated that these technologies will gain wider use in kidney research in the near future, to provide information complementary to other single-cell datasets.

Bioinformatics Analysis of Immune Cells in DKD

Thanks to advances in bioinformatics, efficient algorithms have accelerated the transformation of all types of omics big data into novel therapeutic targets. CIBERSORT is a robust method for assessing immune cell infiltration and is increasingly used in conjunction with bioinformatics approaches to develop novel diagnostic markers and research immune cell infiltration patterns in tissues. By using CIBERSORT to identify immune cells, infiltration of M1 and M2 macrophages, memory B cells, $\gamma\delta$ T cells, and resting mast cells in DKD glomeruli were found to be increased in DKD glomeruli, while neutrophil and activated mast cell infiltration were relatively reduced,^{118–121} however, the infiltration and state of immune cells, such as NK cells, Tregs and DCs appears to be unstable in DKD glomerular tissues. Yan Jia et al¹²² found an up-regulation of M1 macrophages, CD4⁺ T cells, CD8⁺ T cells, Th2 cells, conventional DCs, and activated DCs, but a down-regulation of Tregs in DKD renal tubulointerstitial tissue. Furthermore, increased expression of VCAM1 in renal tubules and interstitial immune cell infiltration contribute to DKD development. Zheng Ye et al¹²³ analyzed high-throughput RNA-SEQ data (GSE142025) from early and late-stage DKD and discovered that immune spectrum diversity and abundance were significantly increased in patients with late-stage DKD, while immune function in patients with early-stage DKD was not significantly changed. In contrast, immune-related signaling pathways, primarily concentrated in immune cells are significantly activated in early-stage DKD. B cell-mediated antibody responses may play an essential part in DKD pathogenesis.

Through analysis of online single-cell RNA profiles of patients with DKD (GSE131882), B cells, T cells, and plasma cells were identified as involved in DKD progression and may be contribute to crosstalk with TECs.¹²⁴ Cell crosstalk analysis using CellPhoneDB based on GSE131882 suggested that CD20 expression was significantly increased in the DKD group.¹²⁵ Drugs that target antibodies against CD20 or B cells hold promise as potential DKD therapeutic options. As previously reported, the mTOR pathway contributes to DKD pathogenesis.¹²⁶ By analyzing GSE131882, Xi Lu et al also found that the differentially expressed immune cell marker genes (*RICTOR*, *PRKCB*, and *EIF4B*) were significantly enriched in the mTOR pathway and confirmed these results using RT-qPCR and Western blot experiments in DKD rat models.¹²⁷

Bioinformatics analysis has also provided more clues to the interactions between immune cells and DKD. Skewed gene patterns in both particular immune cells in the bloodstream and equivalent immune cells infiltrated in DKD kidneys indicated significant enrichment of Fc epsilon receptor (FcER1) and T-cell receptor-mediated signaling. Elevated FcER1 gene and protein levels were observed in the kidneys of patients with DKD and were co-localized with infiltrating mast cells.¹²⁸ FcER1 activation may lead to release of inflammation mediators from mast cells, thereby exacerbating DKD.

Indoleamine 2,3-dioxygenase 1 (IDO1) was identified as a core immune gene with increased expression in mice with DKD. M1 macrophage and monocyte numbers were positively correlated while resting memory CD4⁺ T cell number was negatively correlated, with IDO1 upregulation. In vitro experiments verified that high glucose stimulation resulted in upregulation of IDO1 expression in peritoneal macrophages and that inhibition of IDO1 reversed the production of inflammatory factors.¹²⁹ Qiannan Xu et al¹³⁰ identified *VCAN* as a hub gene related to immune injury in DKD tubulointerstitial injury, levels of its translated protein, versican, in patient renal tissues are associated with immune cell accumulation in the kidneys. CCL19 levels were increased in DKD kidneys and HK-2 cells treated with high glucose, where CCL19 is considered a core molecule in DKD, able to regulate lymphocyte circulation, as well as B- and T-cell migration in the thymus and secondary lymphoid organs, through binding to the CCR7 receptor.¹³¹ Xiaohui Li et al¹³² identified *CASPI*, *MS444A*, *CD53*, and *GBP2* as hub genes implicated in macrophage-mediated inflammation in DKD. Further experiments showed that GBP2 can promote DKD progression by activating pro-inflammatory M1 macrophages via the notch1 signaling pathway.

Meng Zhou et al¹³³ discovered that various signaling pathways, such as those involving VISTANT, SPP1, and IGF, as well as receptor-ligand pairs, including NRG1-ERBB4, SPP1-CD44, Igf1-Igf1r, and NAMPT-INSR, may play significant roles in DKD by inducing crosstalk between renal and immune cells. Hypoxia-related immune molecules, including PSMB8, PSMB9, RHOA, VCAM1, and CDKN1B, may regulate T cells after tubular lesions, whereas APOID1, TGFBR3, KDR, and CPEB1 may influence T cells after glomerular injury.¹³⁴ *LCK* and *HCK* were identified as two core genes, levels of which were increased in DKD. Correlation analysis revealed that both were associated with active DC, CD8⁺ T cells, CD4⁺ T effector memory, CD8⁺ T effector memory, and mast cells.¹³⁵ Xueqin Zhang et al¹³⁶ identified *SLIT3*, *PDE1A*, and *CFH* as hub genes closely associated with DKD, which are significantly positively correlated with $\gamma\delta$ T cell, M2 macrophage, and resting mast cell levels. Mingming Xu et al¹³⁷ identified three immunological and oxidative stress-related hub genes (*CD36*, *SLC1A3*, and *ITGB2*) by merging weighted gene co-expression network analysis (WGCNA), protein-protein interaction (PPI) networks, and machine learning data; however, because their results were based solely on bioinformatics analysis, with no basic experiments to validate them, it is unclear whether the conclusions are truly valid.

Biomarkers linked to immune infiltration provide a fresh perspective to inform future investigations of DKD diagnosis and therapy options. Jing Chen et al¹³⁸ identified six pro-inflammatory and pro-fibrotic candidate targets (including CCR2, MOXD1, COL6A3, COL1A2, PYCARD, and C7) as potential DKD susceptibility genes and validated them by RT-qPCR in kidney samples from DKD mice. Iron concentration changes are intimately related to the variety and complexity of the immunological milieu in patients with DKD, and The Iron Death Scoring System can establish links between iron content and immune cell infiltration. To this end, JingYuan Ma et al¹³⁹ identified the iron death core genes (*PRDX6*, *DUSP1*, *PEBP1*, *GABARAPL1*, *ZFP36*, *RGS4*, and *TSC22D3*) as key factors in iron concentration and immune inflammation, and these genes reliably distinguished DKD from control samples. Wang Yuejun et al¹⁴⁰ used VEGFC, FN1, and complement component 3 as immunological biomarkers of DKD, and found that they were positively correlated with M2 macrophage levels. Clinical database verification demonstrated that, VEGFC is a significantly correlated with eGFR in patients with DKD. Juan Jin et al⁷³ identified biomarkers, including CTLA-4, CXCR3, CCR4, CD39, PD-1, and HLA-DR, via mass cytometry (CyTOF) analysis, which are associated with significantly altered monocyte, T cell, and B cell subpopulations in early-stage DKD; however, further validation in other cohorts is necessary. Shaojie Fu et al¹²¹ used three different algorithms, including least absolute shrinkage and selection operator (LASSO), support vector machine-recursive feature elimination (SVM-RFE), and random forest (RF), to identify DUSP1 and PRKAR2B as possible biomarkers. Further, immunohistochemical staining indicated that DUSP1 and PRKAR2B expression levels were significantly decreased in patients with DKD. Moreover, expression of these two genes was negatively correlated with M2 macrophage infiltration and positively correlated with neutrophil infiltration. By applying the Venn diagram and machine learning algorithms (LASSO, RF, and SVM-RFE), Han et al¹⁴¹ found that PRKAR2B and TGFBI are potential diagnostic biomarkers for glomerular injury related to immune cell infiltration. These algorithms were also used to demonstrate that ADI1, PTGS2, DGKH, and POLR2B, which are associated with immune infiltration, have potential to serve as diagnostic biomarkers for DKD.¹⁴² Decreased expression levels of ADI1 and PTGS2 in patients with DKD are associated with enhanced immune system activation. The significance of DGKH and POLR2B in DKD requires further validation. Handi Zhou et al¹⁴³ identified CCR2, CX3CR1, and SELP as potential diagnostic

biomarkers for DKD, which were associated with checkpoints, cytolytic activity, macrophages, MHC class I molecules, and paraneoplastic inflammation.

Although bioinformatics analyses have yielded various results, small sample sizes and database limitations mean that the findings often require validation using basic experimental methods.

Conclusion

In summary, immune cell imbalance can cause disorders of the immune-inflammatory microenvironment through multiple signaling pathways, resulting in permanent kidney damage and the development of DKD. Therefore, further understanding of the molecular mechanisms underlying immune cell function in the context DKD is of great importance for improving renal disease. In recent years, the development of single-cell sequencing and bioinformatics technologies, to assess gene expression patterns in individual cells in populations of different origins, have facilitated better understanding of the dynamic evolution of immune cells in the kidney and analysis of the complex underlying DKD at the cellular level, providing new approaches to clinical diagnosis and treatment.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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