Inflammation in Diabetic Kidney Disease

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Keywords
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Abstract

\textbf{Background:} Diabetes is a growing public health problem. Diabetic kidney disease (DKD) is the most prevalent chronic renal disease and the major cause of end-stage renal failure worldwide, predominantly due to the increase of Type 2 diabetes associated with obesity [1]. DKD has been classically considered the consequence from the interaction between hemodynamic and metabolic factors, but current knowledge indicates that its pathogenesis is multifactorial, where the immune response and inflammation play a major role [2, 3]. In addition, pro-inflammatory signaling pathways and their downstream products are emerging as new biomarkers and promising therapeutic targets [4].

\textbf{Inflammation in DKD}

Diabetes mellitus (DM) is associated with hemodynamic and metabolic alterations that produce the activation of diverse transduction pathways in virtually all types

Introduction

Diabetic kidney disease (DKD) is the most prevalent chronic renal disease and the major cause of end-stage renal failure worldwide, predominantly due to the growing incidence of Type 2 diabetes associated with obesity [1]. DKD has been classically considered the consequence from the interaction between hemodynamic and metabolic factors, but current knowledge indicates that its pathogenesis is multifactorial, where the immune response and inflammation play a major role [2, 3]. In addition, pro-inflammatory signaling pathways and their downstream products are emerging as new biomarkers and promising therapeutic targets [4].

of kidney cells [2, 3]. DKD is associated with both systemic and local renal inflammation with the participation of crucial inflammatory cells, molecules, and pathways, such as macrophages, the nuclear transcription factor-kappa B (NFkB), the janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway, and inflammatory cytokines [2, 3].

The mononuclear phagocyte system is activated in DM. Macrophages infiltrate the kidney, and the cycle of cytokine release and monocyte and macrophage recruitment culminates in inflammatory-related structural changes. Other cells such as mast cells also infiltrate the tubule-interstitium and releases inflammatory mediators and proteolytic enzymes. The magnitude of macrophage infiltration and the extent of mast cell degranulation is associated with the rate of loss of estimated glomerular filtration (eGFR) [4].

NFkB, a transcription factor that is activated by cytokines and oxygen radicals, controls the expression of genes involved in different processes, such as the immune response and inflammation. In addition, NFkB is central in the interplay among the different factors, molecules, and pathways resulting in structural alterations and functional abnormalities observed in DKD, such as activation of the renin-angiotensin system, advanced glycation end-products accumulation, and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH)-dependent oxidative stress [2, 3].

**Inflammatory Cytokines**

Cytokines are a group of polypeptide signaling molecules that promote autocrine, paracrine, and juxtacrine signaling as a part of the innate immune response. Production of cytokines is induced by numerous stimuli in DM [4]. In the kidney, both blood-borne cells as well as diverse intrinsic renal cells (glomerular, endothelial, tubular, and mesangial cells) are able to synthesize inflammatory cytokines. The levels of these substances increase as nephropathy progresses, with an independent relationship between these inflammatory parameters and urinary albumin excretion, suggesting a role in the pathogenesis of DKD [2, 3].

Different actions from diverse cytokines, such as interleukin (IL)-1, IL-6, IL-18, and tumor necrosis factor-alpha (TNFa) have been involved in the pathogenesis of DKD [5, 6]. IL-1 stimulates the production of prostaglandin E and the release of phospholipase A2, participating in the development of intraglomerular hemodynamic abnormalities. IL-1 activity is also linked to increased permeability of vascular endothelial cells. IL-6 plays a role in facilitating neutrophil infiltration of the tubule-interstitium, influences extracellular matrix dynamics, and promotes overall kidney hypertrophy, thickening of the glomerular basement membrane, podocyte hypertrophy, and cell cycle arrest, which is correlated with albuminuria. IL-18 stimulates the release of interferon gamma and other cytokines, increases the expression of adhesion molecules, and induces endothelial cell apoptosis. High serum levels of IL-18 have been noted in patients with macroalbuminuria, suggesting a role in the development of microvascular kidney complications. Early in the course of diabetes, both glomerular and tubular cells show increased TNFa mRNA expression levels. TNF-a has multiple actions: induction and differentiation of inflammatory cells, cytotoxicity to kidney cells, activation of apoptosis, altered glomerular hemodynamics, increased vascular endothelial permeability, and increased oxidative stress. TNFa exerts its biological actions via the interaction with 2 cell surface receptors, TNFa receptor 1 (TNFR1) and 2 (TNFR2), which have shown a promise value as new prognostic DKD biomarkers [7].

**Chemokines**

Chemokines are a subgroup of cytokines that function as “chemoattractant” molecules, playing a key role in inflammatory cell recruitment, migration, and interaction, as well as in cellular adhesion, differentiation, and tissue damage in the setting of DKD [8]. Several inflammatory chemokines, which are upregulated in response to metabolic and hemodynamic features of the diabetic milieu, participate in the pathogenesis of renal damage in diabetes, particularly monocyte chemotactic protein-1 (MCP-1)/chemokine C-C motif ligand 2 (CCL2), C-X3-C motif chemokine (CX3CL1), and CCL5/RANTES (C-C motif ligand 5/regulated on activation, normal T cell expressed and secreted).

Elevated levels of MCP-1/CCL2 have been reported in biopsied kidneys and urine from patients with DKD, and they play a role in macrophage infiltration of the tubule-interstitium. Angiotensin II directly induces MCP-1/CCL2 expression, and blockade of the renin-angiotensin system (RAS) leads to the reduction of MCP-1/CCL2 in urine. Other biological roles for MCP-1/CCL2 include effacement of podocyte foot processes, podocyte injury with glomerular basement membrane denudement, and damage to the slit diaphragm, all of which explain the correlation between urine levels of MCP-1/CCL2 and albuminuria. The receptor for this chemokine (MCP-1/CCL2-C chemokine receptor type 2 [CCR2]) is distributed in the kidney mononuclear phagocyte system and possibly in differentiated podocytes. CX3CL1 and CCL5/RANTES
are upregulated in diabetes, both within the glomerular and tubular cells, and in peritubular capillaries, acting as chemoattractants for immune cells and for cellular adhesion.

**Adhesion Molecules**

Intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion protein 1, endothelial cell-selective adhesion molecule, and E-selectin are cell surface-localized factors that facilitate intercellular binding, adhesion, and intercellular communication, participating in the pathogenesis of DKD [9]. The expression of adhesion molecules in DKD is increased in response to TNFa, NFkB, and hemodynamic shear stress. ICAM-1 exhibits high expression in resident kidney cells, and elevated urine levels of ICAM-1 have been related to DKD progression. High circulating levels of soluble forms of vascular cell adhesion protein 1 and ICAM-1 are also related to the progression of DKD from microalbuminuria to macroalbuminuria. Endothelial cell-selective adhesion molecule reduces glomerular permeability, and its downregulation in early DKD may promote albuminuria, whereas soluble levels of E-selectin are positively correlated with albuminuria and with cardiovascular disease.

**Inflammatory Signal Transduction**

JAK/STAT is an intracellular cytokine-associated signaling pathway that serves as a key mediator between paracrine stimuli and nuclear receptors. Cytokines and diabetic factors activate this essential mechanism that regulates cell activation, proliferation, recruitment, migration, and differentiation [10]. The upregulation of JAK/STAT has been reported in the glomerular cells of patients with early DKD, and tubule-interstitial expression of various JAK and STAT isoforms increases with disease progression and correlates inversely with the eGFR.

NF-kB, a key transcription factor for inflammatory processes in the diabetic kidney, is activated via JAK/STAT. In resident kidney cells, NF-kB is rapidly activated by diverse stimuli, including hyperglycemia, advanced glycation end-products, mechanical stress, reactive oxygen species (ROS), inflammatory cytokines, angiotensin II, and albuminuria/proteinuria. Upon activation, NF-kB stimulates the transcription of proinflammatory cytokines, chemokines, and adhesion molecules [6].

JAK/STAT members are controlled in a classical negative-feedback loop by the suppressors of cytokine signaling (SOCS) family (SOCS1–7 and CIS). The dysregulated JAK/STAT/SOCS pathway contributes to the pathogenesis of cancer, autoimmune and inflammatory disorders including diabetes. Moreover, the activation of JAK/STAT is an important mechanism by which hyperglycemia contributes to renal damage [11].

**NADH Oxidase and Suppressors of Cytokine Signaling**

Chronic hyperglycemia in combination with growth factors and cytokines act on cells to impair redox balance due to either increased generation of ROS or insufficient antioxidant defense systems, thus resulting in oxidative damage of biological macromolecules (lipids, proteins, and DNA) and tissue injury. Potential sources of ROS include NADPH oxidase (Nox), a multi-subunit enzyme that catalyzes the generation of the reactive-free radical superoxide by reduction of O2 using either NADPH or NADH as a substrate. Collectively known as the Nox family, 7 isoforms are expressed in mammals, Nox1 and Nox4 being the main isoforms involved in DKD [12].

**Therapies Targeting Inflammation: Future Perspectives**

New therapeutic strategies in DKD have moved toward anti-inflammatory agents as potential new treatments [13] (Table 1).

Pentoxifylline (PTF) is a methylxanthine-derived phosphodiesterase inhibitor with beneficial effects on microcirculatory blood flow. A meta-analysis published in 2008 reported a substantial antiproteinuric effect of PTF in patients with diabetic nephropathy, and pointed to the reduction of proinflammatory cytokines as the most likely explanation for this action [14]. The Pentoxifylline for Renoprotection in Diabetic Nephropathy study [15] was a clinical trial that evaluated the effect of PTF in DKD patients with residual albuminuria despite RAS inhibitor treatment. After 24 months, the eGFR decreased by 2.1 ± 0.4 mL/min/1.73 m² in the treatment group, a significant difference (p < 0.001) compared with 6.5 ± 0.4 mL/min/1.73 m² decline in the control group. The between-group difference in the change in albuminuria between the groups was also significant (5.7 vs. −14.9%; p = 0.001). Interestingly, a recent post hoc analysis of the Pentoxifylline for Renoprotection in Diabetic Nephropathy trial have shown that treatment with PTF leads to a significant increase in serum and urinary Klotho concentrations, clinical findings supported by experimental data that show that PTF prevents the downregulation of Klotho protein and mRNA expression induced by albumin and inflammatory cytokines in renal tubular cells [16].

Emapticap pegol is an MCP-1/CC2 antagonist evaluated in a phase 2, placebo-controlled trial of DKD pa-
patients with residual macroalbuminuria while on RAS inhibitor therapy. After 12 weeks, urinary albumin-to-creatinine ratio (UACR) was lower by 29% compared with baseline, without differences from the placebo group. The maximum difference, that is, 26% ($p = 0.06$) between empaticap and placebo, was seen 8 weeks after discontinuation of treatment [17].

CCX140-B is a selective inhibitor of CCR2 that was tested in a double-blind, placebo controlled trial also enrolling DKD patients with proteinuria, eGFR of 25 mL/min/1.73 m$^2$ or higher, and managed on RAS inhibitors, who were treated with 5- or 10-mg doses of CCX140-B. Only participants who had uninterrupted treatment for 52 weeks were included in the intention-to-treat analysis. Treatment with the 5-mg dose resulted in a significant decrease in albuminuria of 18% as compared to the placebo group. The albuminuria-lowering treatment effect persisted throughout the 52 weeks of study [18].

Baricitinib, a JAK 1/2 inhibitor, reduced UACR in a dose-dependent manner after 3 and 6 months of treatment in a phase 2 clinical trial in DKD patients with residual macroalbuminuria on RAS inhibitor treatment. A daily dose of 4 mg Baricitinib decreased morning UACR by 41% at week 24 compared with placebo; there was no change in eGFR but decreased serum levels of inflammatory biomarkers including urine C-X-C motif chemokine-10 and urine C-C motif ligand-2, plasma soluble TNFR 1 and 2, ICAM-1 and serum amyloid A [19].

Studies have shown that the overexpression of SOCS in the kidney can relieve the progression of DN by inhibiting the JAK/STAT pathway. Moreover, SOCS can also suppress renal tubular epithelial-mesenchymal transition that is induced by oncostatin-M, a multifunctional member of the IL-6 cytokine family. Finally, renal delivery of SOCS1 and SOCS3 ameliorates proteinuria and leads to reduced oncostatin expression and extracellular matrix deposition [11, 20].

**Conclusions**

DM is a major global health problem and DKD is one of its most important complications. Conventional treatments provide incomplete kidney protection and new therapeutic approaches are needed. Nowadays, inflammation is acknowledged as a key factor in the develop-

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Identifier</th>
<th>Study population</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentoxifylline</td>
<td>Inflammatory Cytokines</td>
<td>EudraCT number 2007-005985-10</td>
<td>Type 2 diabetes eGFR 15–60 UAE &gt;30 mg/24 h</td>
<td>Mean difference in UAE of 21% ($p &lt; 0.001$) and eGFR decline 4.3 mL/min/1.73 m$^2$ lower than in the placebo group ($p &lt; 0.001$)</td>
</tr>
<tr>
<td>Baricitinib</td>
<td>JAK1/JAK2</td>
<td>NCT01683409</td>
<td>Type 2 diabetes Macrolalbuminuria eGFR 20–75 mL/min/1.73 m$^2$</td>
<td>Albuminuria reduction by 40% No effect on eGFR</td>
</tr>
<tr>
<td>Emanticap Pegol (NOX-E36)</td>
<td>CCL2</td>
<td>NCT01547897</td>
<td>Type 2 diabetes eGFR &gt;25 mL/min/1.73 m$^2$ UACR &gt;100 mg/g</td>
<td>Albuminuria reduction by 29% compared with baseline ($p &lt; 0.05$), but no significant difference with placebo</td>
</tr>
<tr>
<td>CCX 140-B</td>
<td>CCR2</td>
<td>NCT01447147</td>
<td>Type 2 diabetes eGFR 25–25 mL/min/1.73 m$^2$ UACR 100–3,000 mg/g</td>
<td>18% reduction of albuminuria compared with placebo ($p &lt; 0.0004$) in the 5 mg group. No reduction of albuminuria in the 10 mg group</td>
</tr>
<tr>
<td>CTP-499</td>
<td>PDE</td>
<td>NCT01487109</td>
<td>Type 2 diabetes eGFR no limit UACR 300–5,000 mg/g</td>
<td>16% UACR reduction</td>
</tr>
<tr>
<td>LY3016859</td>
<td>TGF-α/epiregulin</td>
<td>NCT01774981</td>
<td>eGFR &lt;90 mL/min/1.73 m$^2$ UACR &gt;400 mg/g</td>
<td>Study ongoing. No results available</td>
</tr>
</tbody>
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eGFR, estimated glomerular filtration rate; TGF-α, transforming growth factor alpha; UACR, urinary albumin-to-creatinine ratio.
ment and progression of DKD, and therefore, anti-inflammatory targets directed at specific molecular signatures can be promising therapeutic strategies for DKD.

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**Disclosure Statement**

The authors have no conflicts of interest to declare.

**References**