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RELEVANCE OF TNF-α IN THE CONTEXT OF OTHER INFLAMMATORY CYTOKINES IN THE PROGRESSION OF DIABETIC NEPHROPATHY

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Abstract

An ancillary paradigm that has evolved recently in the pathogenesis of diabetic nephropathy includes subclinical “micro-inflammation” with influx of macrophages and consequential generation of myriad pro-inflammatory cytokines and ensuing kidney damage. Among various pro-inflammatory cytokines TNF-α has attracted the most attention since it amplifies the inflammatory network of cytokines leading to worsening of the progression of diabetic nephropathy. The accompanying article by Awad *et al*. examines the role of TNF-α in the pathogenesis of experimental diabetic nephropathy.

Keywords

Diabetic Nephropathy; Inflammatory Cytokines

Diabetic nephropathy (DN) is the major prototypic example of reno-vascular complications of diabetes, and it is the leading cause of end stage of kidney disease (ESRD) in the Western population and in developing countries. It has reached epidemic proportions worldwide and is an important cause of morbidity and mortality in 30% of diabetic patients, and thus there is an urgent need for the development of effective therapies. Although many therapeutic interventions, including reducing hyperglycemia and intra-glomerular pressure, have been shown to slow down the progression of DN many patients still develop ESRD. This may be due to the fact that damage to various renal compartments due to diabetes may be multifactorial and the processes involved may be intricately interwoven and difficult tease out to treat them individually in an effective manner. Recently, attention of several investigators has been drawn to control subclinical chronic inflammation (micro-inflammation) in the kidney, which likely plays a critical role in promoting progression of

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diabetic nephropathy.[1, 2] In this regard Awad *et al*. (Current issue of KI, 2015) reported effectiveness of anti-tumor necrosis factor-alpha (TNF-α) antibody in the amelioration of experimental diabetic nephropathy in *Ins2*^{Akita} diabetic mice.[3] Likewise they observed protection from streptozotocin (STZ)-induced hyperglycemic renal injury in another mice model deficient in macrophage-specific TNF-α (*CD11b*^{Cre}/*TNF*-α$^{Flox/Flox}$). In both models, besides reduced levels of tumor necrosis factor-α (TNF-α) the authors noted improvement in renal function and morphology, and reduced macrophage influx associated with down-regulation of inflammatory cytokines, including monocyte chemoattractant protein-1/ chemokine (C-C motif) ligand 2 (MCP-1/CCL2) which is involved in recruitment of monocytes. These novel findings reinforce the importance of previous studies that have elucidated the roles of inflammatory cytokines in orchestrating a complex series of signaling events resulting in renal injury in diabetes. We will therefore briefly discuss the importance of the current study in the context of previous research.

Hyperglycemia-induced metabolic perturbations and hemodynamic disturbances have been considered the main factors promoting diabetic renal injury for the past 2-3 decades. The hyperglycemia-induced metabolic derangements coupled with channeling of glucose intermediaries into polyol and hexasoamine pathways lead to the activation of protein kinase C (PKC), generation of advanced glycation end-products (AGEs) and reactive oxygen species (ROS). These factors lead to activation of renin-angiotensin-aldosterone system (RAAS) that dramatically worsens the progression of diabetic nephropathy.[4] During the past decade the role of innate immunity and micro-inflammation have emerged as an important factors in causing of diabetic renal injury.[1] The evolution of this concept stemmed from studies in the late 1980s and early 1990s indicating that the AGEs and AGEs-modified glomerular basement membranes (GBMs) are capable of stimulating and inducing secretion of TNF-α and interleukin-1 beta (IL-1β) by macrophages, which express the receptor for AGEs.[5] This set the stage for investigators to explore the role of inflammatory cytokines in progression of diabetic nephropathy in humans and in various animal model systems.

More than 2 decades ago Furuta *et al*. demonstrated an important role for macrophages in diabetic nephropathy since they observed a transient influx of macrophages predominantly into glomeruli which declined in advanced stages of diabetic glomerulosclerosis.[6] This matter was later revisited, and now there is growing evidence that the macrophages are indeed the major inflammatory cells infiltrating the diabetic kidney in the setting of subclinical chronic inflammation associated with diabetic renal injury. After accumulating in glomeruli and/or the interstitium, macrophages initiate a series of events involving complex interactions with resident kidney cells inducing them to secrete pro-inflammatory cytokines that ultimately results in diabetic kidney injury. In diabetic *milieu*, factors including hyperglycemia, AGEs, oxidized-low density lipoprotein (LDL) and ROS and as well as angiotensin II (Ang II) can activate resident kidney cells *via* inducing the stress-activated protein kinases (SAPKs), *i.e*., P38 MAPK and JNK; which increases secretion of chemokines including MCP-1 and CSF-1 and expression of adhesion molecules, such as, ICAM/VCAM.[7] These signaling cascades facilitate the recruitment of monocytes and T-lymphocytes which, in conjunction with renal parenchymal cells, elaborate pro-inflammatory cytokines and ROS in an autocrine and paracrine manner, thereby establishing

a vicious cycle of inflammatory injury in the glomerular and tubulo-interstitial compartments of the kidney. The most important inflammatory cytokines that are induced in this setting include TNF-α, IL-1, IL-6 and IL-18. There is also increased activation of transcription factor NF-κB, a master regulator of innate immunity, which contributes to further progression of diabetic nephropathy.[8]

The evidence for the M1 type macrophage influx and cytokine-induced inflammatory damage in the setting of hyperglycemia is derived from both clinical as well as experimental studies. In humans, accumulation and activation of macrophages in kidneys of diabetic patients positively correlates with severity of hyperglycemia, plasma creatinine, and hemoglobin A1c, and the degree of albuminuria and glomerular and tubular damage.[1] Likewise the serum levels of inflammatory cytokines, *e.g*., TNF-α, were positively correlated with macro- and micro-albuminuria; however, a recent study refutes these findings.[8] Nevertheless, experimental animal data convincingly supports the notion that macrophage-induced signaling correlates with severity of renal injury in hyperglycemia. For instance, *db/db* mice deficient in adhesion molecule ICAM have reduced renal monocyte influx, albuminuria, glomerular hypertrophy and tubular damage.[1, 2] These investigators also reported decreased accumulation of glomerular and interstitial macrophages and attenuated renal fibrosis in CCL2/MCP$^{-/-}$ mice with streptozotocin induced diabetes. Similar results have been reported with the ablation CCR2, the receptor for MCP-1/CCL2. CCR2$^{-/-}$ mice were noted to have attenuated albuminuria, glomerular macrophage influx and ECM expression.[3] Since MCP-1 pathobiology in the context of diabetic nephropathy is well-established and MCP-1 and TNF-α are central to the pathogenesis of inflammatory damage in hyperglycemia Awad *et al*. further explored the relevance of TNF-α in diabetic injury in the current study.[3]

TNF-α is a transmembrane homotrimeric protein with molecular mass of 34-kDa, and after cleavage by the tumor necrosis factor-α converting enzyme 17 (ADAM-17) is released into the circulation and binds to TNF receptor 1 (TNFR1) or TNFR2 and modulates a complex series of immune and inflammatory responses.[8, 9] Almost all resident kidney cells, including mesangial cells, podocytes, endothelial and tubular cells are capable of producing TNF-α. However, TNF-α is predominantly synthesized by infiltrating T-cells and monocytes/ macrophages, where hyperglycemia and AGEs serve as potent inducers of TNF-α in the resident kidney cells. TNF-α can amplify the renal inflammatory response by increasing the expression of adhesion molecules and by inducing the cells to release other cytokines, growth factors and pro-inflammatory chemokines, IL-8, MCP-1 and macrophage-colony stimulating factor (M-CSF) in an autocrine and paracrine manner. Since the infiltrating and intrinsic glomerular cells can synthesize TNF-α both can be considered equally important in the causation and perpetuation of glomerular injury. In addition, TNF-α can directly activate NADPH oxidase leading to local generation of ROS *via* phosphodiestrase-dependent mechanisms. Overall, it seems that TNF-α is a prime inducer and driver of renal micro-inflammation and thus is believed to play a central role in the network of pro-inflammatory molecules during the progression of diabetic nephropathy (Figure 1).

TNF-α was hypothesized to play a pathogenetic role in the progression of diabetic nephropathy by Hasegawa *et al*. more than 2 decades ago.[5] Since then many investigators

have attempted to delineate the mechanism(s) by which macrophage derived TNF-α causes renal injury in diabetic *milieu*.[8, 9] In this regard, a number of mechanisms have been proposed by which TNF-α may directly induce renal injury or indirectly *via* the elaboration of other inflammatory cytokine. TNF-α may stimulate the production of endothelin-1, leading to dysregulation of vascular tone resulting in reduced intra-renal blood flow and glomerular filtration rate. It may also disrupt endothelial intercellular junctions and thus adversely affect the integrity of glomerular filtration barrier thereby causing increase in its permeability and albuminuria. In addition, TNF-α may also have direct cytotoxic effects on glomerular podocytes and tubular cells and thus may induce apoptosis and cell death. Alternatively, it may cause activation of protein kinase/phosphatidylinositol-3 kinase pathway or activation of NADPH oxidase culminating into the generation of ROS and consequential cellular damage. With respect to glomerular podocytes, it may reduce the expression of nephrin *via* activation of PI3K/Akt pathway, and decrease Akt activity in the setting of diabetes leading to reduced cell survival. Lastly, it may synergize with the actions of another pro-fibrogenic cytokine, TGF-β, in promoting the ECM accumulation by increasing the expression of fibronectin and TIMP-1.[8] These myriad effects of TNF-α support its role as an inducer as well as a driver of renal injury by modulating the expression of other downstream cytokines. In line with this notion are the findings of the current study in which inhibition of TNF-α led to decreased expression of other cytokines, *i.e.*, MCP-1, keratinocyte derived cytokine (KC) and granulocyte-macrophage colony stimulating factor (GMCSF), along with improvement in renal functions and reduction of urinary albumin excretion.[3]

Despite the large literature on the pathogenetic roles of inflammatory cytokines in the pathogenesis of diabetic nephropathy, studies need to determine whether TNF-α is a suitable biomarker or target for therapeutic intervention to ameliorate the progression of diabetic nephropathy. Many studies have reported a relationship between elevated serum and urinary levels of TNF-α and abnormal urinary protein excretion (UAE) and reduced GFR. Increased expression of TNF-α in both glomerular and tubulo-interstitial compartments have also been reported in patients with diabetic nephropathy and in murine models of experimental diabetes. Furthermore, elevated concentrations of circulating TNF-α, TNFR1 and TNRF2 are associated with loss of renal function.[9] However, a recent report indicates a positive correlation only of urinary, but not serum TNF-α, with severity of microalbuminuria in type 2 diabetes.[8] The issue if TNF-α/TNF-α receptor system could serve as a therapeutic target for the deceleration of diabetic nephropathy in diabetic mice or patients has been addressed in several investigations. [2, 3, 8, 9] Like the current study, other studies have also documented a reduction of urinary albumin excretion and improvement in renal functions following the administration of monoclonal antibody directed against TNF-α (infliximab), or circulating receptor fusion protein (etanercept) or antagonist of TNF-α receptor (progranulin). Another anti-inflammatory small molecule inhibitor, pentoxyphyline (PTF), has been shown to slow the progression of diabetic nephropathy.[2] PTF is a methylxanthine derived phosphodiestrase inhibitor that reduces TNF-α gene transcription. Administration of PTF was shown to reduce albuminuria and eGFR, most likely, by improving the local renal micro-circulatory fluid dynamics. In addition to above mentioned pharmacological interventions the new information provided in the current study indicates that genetic ablation of TNF-α also leads

to amelioration of diabetic injury. Finally, in view of the above discussion of the vast amount scientific and clinical work encompassing past 2-3 decades one can certainly make a timely comment at this juncture that the TNF-α/TNF-α receptor system certainly has some added value in serving as a potential therapeutic target besides controlling hyperglycemia and hypertension to improve the outcomes in the progression of diabetic nephropathy towards ESRD.

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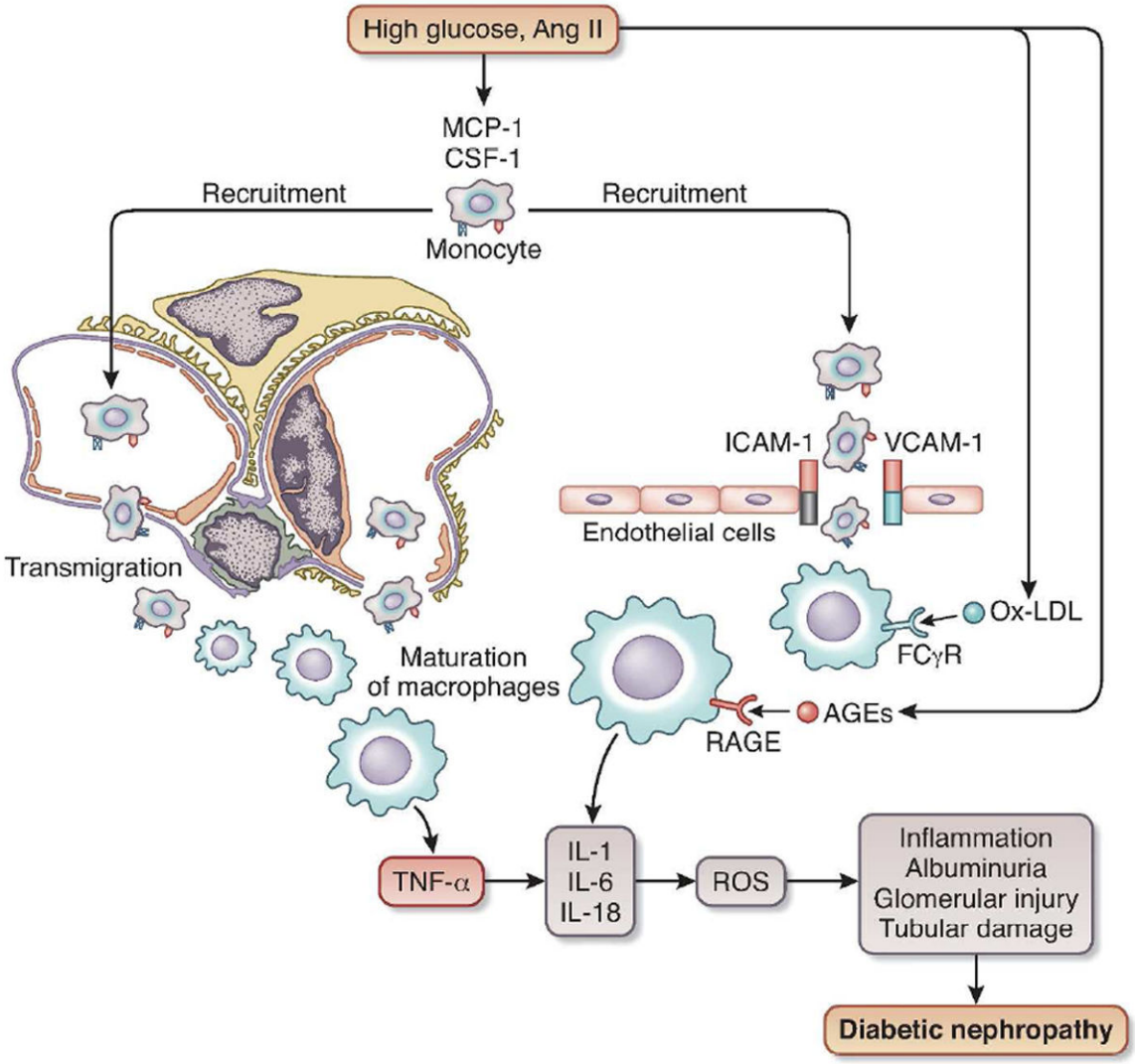

Figure 1.

Schematic drawing depicting sequence of events relevant to inflammatory damage in the kidney during progression of diabetic nephropathy. High glucose, advanced glycation end products (AGEs), Ox-LDL and angiotensin II (Ang II) induce expression of monocyte chemo-attractant protein-1 (MCP1) and colony stimulating factor-1 (CSF-1) and cell adhesion molecules (ICAM-1 and VCAM-1) in various renal cellular compartments, which enhance recruitment of monocyte/macrophages in the kidney under high glucose ambience. In addition, up-regulated expression of MCP-1 and CSF-1 in renal cells promotes trans-endothelial migration of macrophages. Furthermore, AGEs bind to its receptor RAGE, expressed on the macrophage surface, which promotes activation and maturation of monocyte/macrophage and induces the release of pro-inflammatory cytokine (TNF-α) and over-expression of TNF-α. This further stimulates expression of other cytokines (IL-1, IL-6, IL-18), which consequentially stimulate production of reactive oxygen species (ROS)

leading to subclinical chronic inflammation (micro-inflammation), glomerular and tubular damage and ultimately albuminuria.