REVIEW

Cardiovascular Therapeutics

Relationship between Oxidative Stress and Inflammatory Cytokines in Diabetic Nephropathy

Ahmed A. Elmarakby[1,2,3] & Jennifer C. Sullivan[2,3]

1 Department of Oral Biology, Medical College of Georgia, Augusta, GA, USA
2 Department of Pharmacology & Toxicology, Medical College of Georgia, Augusta, GA, USA
3 Vascular Biology Center, Medical College of Georgia, Augusta, GA, USA

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Correspondence
Ahmed A. Elmarakby, Medical College of Georgia, 1120 15th Street, CL2126 Augusta, GA 30912, USA.
Tel.: (706) 721-2748;
Fax: (706) 721-6252;
E-mail: aelmarakby@mcg.edu

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SUMMARY

The prevalence of diabetes has dramatically increased worldwide due to the vast increase in the obesity rate. Diabetic nephropathy is one of the major complications of type 1 and type 2 diabetes and it is currently the leading cause of end-stage renal disease. Hyperglycemia is the driving force for the development of diabetic nephropathy. It is well known that hyperglycemia increases the production of free radicals resulting in oxidative stress. While increases in oxidative stress have been shown to contribute to the development and progression of diabetic nephropathy, the mechanisms by which this occurs are still being investigated. Historically, diabetes was not thought to be an immune disease; however, there is increasing evidence supporting a role for inflammation in type 1 and type 2 diabetes. Inflammatory cells, cytokines, and profibrotic growth factors including transforming growth factor-β (TGF-β), monocyte chemoattractant protein-1 (MCP-1), connective tissue growth factor (CTGF), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-18 (IL-18), and cell adhesion molecules (CAMs) have all been implicated in the pathogenesis of diabetic nephropathy via increased vascular inflammation and fibrosis. The stimulus for the increase in inflammation in diabetes is still under investigation; however, reactive oxygen species are a primary candidate. Thus, targeting oxidative stress-inflammatory cytokine signaling could improve therapeutic options for diabetic nephropathy. The current review will focus on understanding the relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy to help elucidate the question of which comes first in the progression of diabetic nephropathy, oxidative stress, or inflammation.

Introduction

Diabetic nephropathy is one of the most common microvascular complications of type 1 and type 2 diabetes mellitus and the leading cause of end-stage renal disease worldwide [1,2]. Many factors contribute to the development of diabetic nephropathy including hyperglycemia, hypertension, obesity, a sedentary lifestyle, hereditary, smoking, and advancing age [3,4]. Diabetic nephropathy is characterized by morphological and ultrastructural changes in the kidney including expansion of the molecular matrix and loss of the charge barrier on the glomerular basement membrane [5,6]. The progression from normal albuminuria to microalbuminuria is considered the initial step in diabetic nephropathy which further progresses to macroalbuminuria as renal function continues to deteriorate and glomerular filtration rate (GFR) starts to decline [5,6].

Careful control of glycemic status minimizes the symptoms of diabetic complications indicating that hyperglycemia is the main driving force behind the development of diabetic complications including diabetic nephropathy (Figure 1); however, strict glycemic control is difficult to maintain [7,8]. Current treatment strategies for diabetic nephropathy include glycemic and blood pressure control, low-protein diet, lipid-lowering drugs, and interference with the renin-angiotensin system [9,10]. Although these therapeutic options slow the progression of diabetic nephropathy, the burden and mortality rate of the disease remains very high and the majority of patients with diabetic nephropathy continue to progress to end-stage renal disease. Therefore, more detailed understanding of the molecular mechanisms for disease progression is needed. Both oxidative stress and inflammation are intimately linked with the development of diabetic nephropathy [1,10–12]. Increases in oxidative stress can increase the production of inflammatory

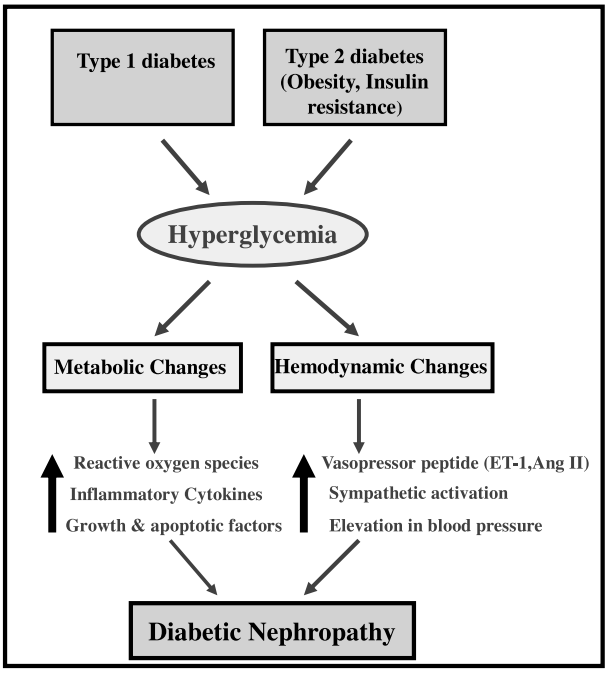

Figure 1 Hyperglycemia is the main sign of type 1 and type 2 diabetes. In turn, hyperglycemia results in a myriad of metabolic and hemodynamic changes that are intimately associated with vascular complications of diabetes including diabetic nephropathy.

cytokines and likewise, an increase in inflammatory cytokines can stimulate the production of free radicals. The goal of this review is to examine how these two pathways interact to contribute to the development of diabetic nephropathy.

Oxidative Stress in Diabetic Nephropathy

Under normal physiological conditions, there is a balance in the generation of oxygen-free radicals and the antioxidant defense mechanisms used to deactivate free radical toxicity [13–15]. Impairment in the oxidant/antioxidant equilibrium results in oxidative stress in numerous pathological conditions including diabetes leading to cellular damage [13–15]. Increasing evidence in both experimental and clinical studies suggests that there is a close link between hyperglycemia, oxidative stress, and diabetic complications [16,17]. Increased oxidative stress in diabetes likely contributes to the pathogenesis of diabetic nephropathy and its progression to end-stage renal disease [18–20]. Enhanced reactive oxygen species (ROS) production in experimental and clinical diabetes have been linked to vasoconstriction, vascular smooth muscle cell growth and migration, endothelial dysfunction, modification of extracellular matrix (ECM) proteins, and increased renal sodium reabsorption [21–24]. The importance of oxidative stress in diabetic nephropathy is underscored by the finding that inhibition of oxidative stress ameliorates the manifestations associated with streptozotocin-induced diabetic nephropathy [21,25]. Streptozotocin selectively targets and kills the beta cells of the pancreas resulting in an experimental model of type 1 diabetes mellitus. In addition, overexpression of human cytoplasmic Cu^{2+}/Zn^{2+} superoxide dismutase (SOD-1) in streptozotocin-induced diabetic transgenic mice attenuates diabetic renal injury [26].

Renal damage and increased levels of oxidative stress are likely related to increased cellular glucose since hyperglycemia induces ROS generation. Reestablishing glycemic control soon after the induction of type 1 diabetes in rats and dogs prevents proteinuria, kidney hypertrophy, and the increase in oxidative and nitrative stress [27,28]. Careful glycemic control also ameliorates microalbuminuria in diabetic patients, and the risk of developing nephropathy is reduced [29]. Recent evidence suggests that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is the primary source of vascular and renal ROS production [30–36]; however, other possible sources include glucose-6-phosphate dehydrogenase, flux through the sorbitol/polyol pathway, and glycation of free amino groups on proteins and amino acids, mitochondrial electron transport enzymes, xanthine oxidase, cyclooxygenase, lipoxygenase, and uncoupled nitric oxide synthase [30,34,37]. For more information regarding the sources and role of oxidative stress in diabetes please see the following reviews [24,37–40].

Inflammation in Diabetic Nephropathy

Diabetic nephropathy has traditionally been considered a non-immune disease; however, recent evidence shows an increase in macrophage infiltration and overproduction of leukocyte adhesion molecules in kidneys from diabetic humans and in experimental animal models of diabetes [41–45]. As a result, there is growing support for the notion that inflammation plays a key role in the pathogenesis of diabetic nephropathy. Leukocytes, monocytes, and macrophages have all been implicated in the process of diabetic nephropathy [41–45] and circulating inflammatory markers and proinflammatory cytokines are strongly associated with the risk of developing of diabetic complications [44–49]. Further support for inflammation to contribute to diabetes comes from studies where immunosuppressive strategies reduce renal macrophage accumulation and attenuate the development of diabetic nephropathy [50–52]. Although several recent reviews have examined the role of inflammatory cytokines in diabetic nephropathy [52–54], the focus of this review is on the interaction of oxidative stress and cytokines in diabetic nephropathy.

Oxidative Stress Stimulates Cytokine Production

Oxidative stress can increase cytokine production via several different mechanisms. Oxygen derivatives, acting as second messengers, activate the transcription factors nuclear factor kappa B (NFκB) and activator protein-1 (AP-1) leading to the transcription of genes encoding cytokines, growth factors, and ECM proteins [55–58]. NFκB is suggested to play an important role in mesangial cell activation leading to renal injury and NFκB expression is increased in kidneys of diabetic experimental animals [59,60]. AP-1 mediates high glucose-induced TGF-β production in mesangial cells and mutation of the AP-1 binding sites on TGF-β abolished high glucose-mediated increases in TGF-β levels [61].

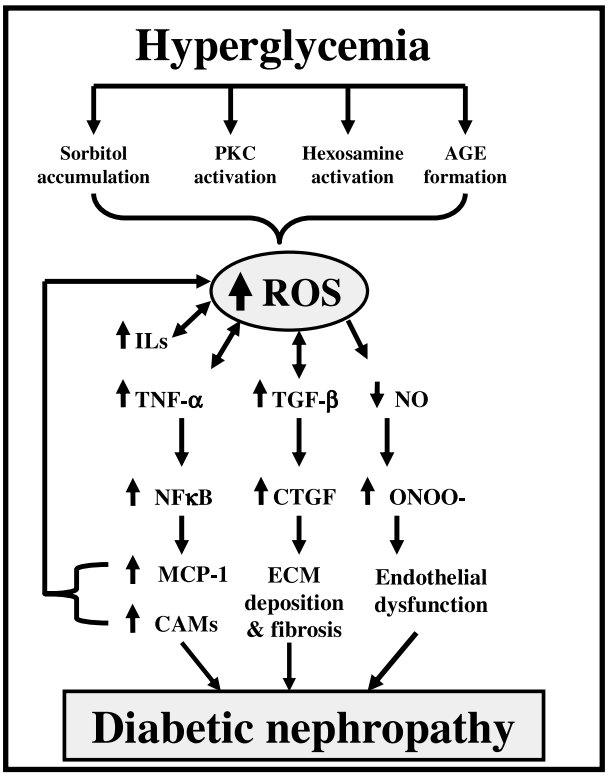

Figure 2 Schematic diagram showing the proposed relationship between hyperglycemia, oxidative stress, and inflammatory cytokines in the pathogenesis of diabetic nephropathy. ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; NFκB, nuclear factor kappa B; MCP-1, monocyte chemoattractant protein-1; CAMs, cellular adhesion molecules; NO, nitric oxide; ONOO–, peroxinitrite; TGF-ß, transforming growth factor-ß; CTGF, connective tissue growth factor; ILs, interleukins.

Peroxynitrite has also been implicated in the enhanced inflammation in diabetes as the result of decreased nitric oxide bioavailability [14,62]. In addition, enhanced macrophage migration in diabetes induces the release of inflammatory and profibrotic cytokines, which further stimulates ROS production [11,63]. Therefore, oxidative stress-induced cytokine production is likely to further increase oxidative stress levels setting up a vicious cycle (Figure 2). The remainder of this review focuses on the relationship between oxidative stress and the most commonly involved cytokines in the progression of diabetic nephropathy.

Transforming Growth Factor-β (TGF-β)

TGF-β is a hypertrophic and fibrogenic cytokine and the causative agent of mesangial expansion and renal insufficiency in human and type 1 experimental models of diabetic nephropathy [64–66]. TGF-β stimulates the deposition of ECM via direct upregulation of matrix protein genes, inhibition of matrix degradation by suppressing proteases, and increasing the synthesis of protease inhibitors, increasing cell surface expression of integrins to promote attachment to newly synthesized matrix, and auto-inducing its own production [67,68]. TGF-β1 mRNA and protein levels are increased in both glomerular and tubular compartments of various rat and mouse experimental models of type 1 and type 2 diabetes [66,69–71]. Direct support for the key role played by TGF-β in the development of diabetic nephropathy comes from studies where treatment with neutralizing monoclonal antibodies to TGF-β prevents glomerular hypertrophy, mesangial matrix expansion, and glomerulosclerosis and preserves renal function in streptozotocin-induced type 1 diabetes and "db/db" type 2 diabetic mice [72,73]. While the role of TGF-β in diabetes is relatively well established, there remains some question as to the stimulus to increase TGF-β levels in the diabetic kidney. We propose that the increase is secondary to a glucose-induced increase in ROS production.

Hydrogen peroxide (H_2O_2) increases TGF-β protein synthesis and stimulates collagen and fibronectin gene expression in cultured human mesangial cells [74]. H_2O_2 also increases TGF-β mRNA and collagen expression in cultured NRK-49F fibroblasts and rat mesangial cells [58]. H_2O_2 effects on ECM protein expression in mesangial cells are directly mediated by TGF-β as incubation of mesangial cells with an anti-TGF-β antibody blocked the effects of H_2O_2 on ECM gene expression [74]. However, H_2O_2 has also been shown to activate NFκB in Jurkat T cells [56]. Therefore, it is possible that the effect of H_2O_2 on TGF-β is mediated by NFκB activation. H_2O_2 is unlikely to be the sole ROS that stimulates TGF-β levels. High glucose-induced increases in TGF-β and fibronectin gene expression in rat mesangial cells is blocked by incubation with SOD and an NADPH oxidase selective inhibitor but not catalase, suggesting that in rat cells superoxide and not H_2O_2 stimulates TGF-β [75]. Incubation of rat glomerular mesangial cells with advanced oxidation protein products (AOPP) increases superoxide, TGF-β, and ECM protein levels, and this effect is blocked by SOD [76]. AOPPs are cross-linking protein products formed during oxidative stress and AOPP levels are increased in plasma and renal homogenates of streptozotocin-induced type 1 diabetic male Sprague-Dawley rats [35]. In diabetic streptozotocin rats, treatment with AOPP further increases oxidative stress and TGF-β levels, both of which were blocked by NADPH oxidase inhibition [35]. Therefore, while evidence supports the hypothesis that increased inflammation in the diabetic kidney is secondary to an increase in oxidative stress, whether this is a direct or an indirect effect remains to be elucidated.

Although data supporting a direct temporal relationship between increased ROS and TGF-β stimulation of ECM overproduction in diabetes *in vivo* is scarce, there is indirect evidence. Antioxidant treatment decreases TGF-β levels in the diabetic kidney. Mice overexpressing SOD1 are protected from streptozotocin-induced increases in renal TGF-β and ECM production compared to diabetic wild-type mice [26] and treatment of streptozotocin diabetic male Wistar rats with SOD-PEG decreases renal 8-hydroxyl guanosine (8-OHdG), a marker of oxidative stress, TGF-β, and fibronectin protein levels [75]. Similarly, treatment with an SOD mimetic inhibits glomerular matrix expansion via the suppression of TGF-β in female Sprague-Dawley rats following streptozotocin injection [76]. The potential importance of SOD in regulating oxidative stress and TGF-β levels in the diabetic kidney were underscored by studies on SOD1 knockout mice, where streptozotocin resulted in greater increases in renal TGF-β in

knockout mice compared to wild-type littermates and this increase was blocked by a SOD mimetic [77]. Thioredoxin has also been implicated as an important determinant of redox balance in diabetes. The thioredoxin system reduces ROS through reversible oxidation of thioredoxin. Transgenic mice over-expressing thioredoxin-1 have less renal damage, lower 8-OHdG excretion, and suppression of TGF-β mRNA following streptozotocin treatment compared to wild-type streptozotocin mice independent of glycemic status [78]. Additional evidence suggests that thioredoxin activity may be suppressed in diabetes. High glucose increases thioredoxin interacting protein (Txnip), an inhibitor of thioredoxin, in proximal tubule HK-2 cells, human aortic smooth muscle cells, and in male Ren-2 and Sprague-Dawley rats following streptozotocin injection [79,80]. In cultured human aortic smooth muscle cells, adenoviral overexpression of thioredoxin reduces glucose-induced ROS while overexpression of Txnip increases ROS. In addition, gene knockdown of Txnip reduces ROS in streptozotocin diabetic male Sprague-Dawley rats [80]. Finally, both vitamin E and taurine supplementation decrease oxidative stress levels and glomerular TGF-β expression in streptozotocin-induced diabetic male Sprague-Dawley rats [81,82]. These data all support the hypothesis that oxidative stress significantly contributes to the increase in TGF-β in diabetic nephropathy (Figure 2).

Connective Tissue Growth Factor

Connective tissue growth factor (CTGF) is a highly profibrogenic molecule which is overexpressed in many fibrotic lesions [83,84]. CTGF is transcriptionally activated by TGF-β and is considered the major downstream effector of TGF-β [83,84]. CTGF is the key factor in stimulating connective tissue cell proliferation, ECM production, and other profibrotic properties of TGF-β [85,86]. CTGF and TGF-β exhibit shared fibrogenic and angiogenic properties *in vivo* as they both promote cell adhesion, migration, proliferation, and differentiation [85,86]. Thus, CTGF plays an important role in collagen production and the maintenance of fibrotic lesions.

Recent studies suggest that CTGF is critically involved in the pathogenesis and progression of diabetic nephropathy [84]. High glucose concentrations and advanced glycation end products stimulate CTGF and TGF-β production in cultured mesangial cells and both are involved in diabetes-induced increases in ECM production, cell migration, and fibrosis [86–89]. CTGF is upregulated in experimental models of type 1 diabetic nephropathy and in diabetic patients [90–93]. In streptozotocin-induced diabetes, urinary CTGF increases during the early development of clinical symptoms then decreases as animals become proteinuric [94]. Urinary CTGF is also elevated in diabetic patients with albuminuria, which is prognostic for the progression to microalbuminuria [95]. Furthermore, inhibition of CTGF signaling using antisense preserves the structure and function of kidney in mouse models of type 1 and type 2 diabetes, underscoring the importance of CTGF in the pathogenesis of diabetic nephropathy [96].

The exact relationship between CTGF and oxidative stress remains to be investigated; however, advanced glycation end products induce oxidative stress and CTGF production [97–99]. In many experimental models of type 1 and type 2 diabetic nephropathy, elevations in oxidative stress parallel elevations in CTGF levels [100–102]. Enhanced renal NADPH oxidase activity in streptozotocin-induced type 1 diabetic mice is associated with increased renal TGF-β, CTGF, and collagen IV expression [102]. However, NADPH oxidase activity and expression are unchanged in diabetic protein kinase C (PKC)-beta knock out mice despite a decrease in renal TGF-β, CTGF, and collagen IV expression [102]. We postulate that increased oxidative stress during diabetes is the trigger for stimulation of TGF-β-CTGF fibrotic signaling (Figure 2).

Interleukins (ILs)

IL-1

IL-1 increases the expression of chemotactic factors and adhesion molecules, enhances vascular endothelial permeability, and stimulates the proliferation of mesangial cells and matrix synthesis [53,54]. IL-1 was first implicated in the development of diabetic nephropathy when glomerular basement proteins isolated from streptozotocin-induced diabetic male rats had significantly greater macrophage, TNF-α, and IL-1 production compared to control rats [103]. IL-1 levels are also elevated in kidneys from male Sprague-Dawley rats treated with streptozotocin compared to control rats [104] and renal expression of IL-1 is significantly correlated with urinary albumin excretion [105].

IL-6

Renal IL-6 expression is positively related to mesangial proliferation, tubular atrophy in diverse models of renal disease, supporting the role of IL-6 in the progression of renal disease [54]. Serum IL-6 levels are significantly higher in patients with type 2 diabetic nephropathy compared to levels observed in diabetic patients without nephropathy suggesting a role for IL-6 in the pathogenesis of diabetic nephropathy [106–108]. In fact, serum IL-6 levels are similar in type 2 diabetic patients with normal albumin excretion and microalbuminuria, but significantly increased in patients with diabetic nephropathy and clinical albuminuria [109]. Serum IL-6 levels are also significantly greater in diabetic patients with overt proteinuria compared to normoalbuminuric and microalbuminuric patients [110,111]. Using high-resolution *in situ* hybridization in kidney biopsies of Japanese patients with diabetic nephropathy, it was found that interstitial expression of IL-6 mRNA correlated significantly with the degree of interstitial injury [112]. Clinical reports are in agreement with studies in experimental animals where renal expression of IL-6 mRNA is increased in streptozotocin diabetic rats compared to controls and levels are significantly associated with urinary albumin excretion [105]. These data support a role for IL-6 in the progression of diabetic nephropathy in the later stages of the disease.

IL-18

IL-18 is a potent inflammatory cytokine secreted from activated monocytes/macrophages and it is known to induce interferon-γ (IFN-γ), which increases functional chemokine receptor expression in human mesangial cells [53]. IL-18 also stimulates the production of other inflammatory cytokines including IL-1, TNF-α,

and IL-6, upregulates ICAM-1 expression, and induces endothelial cell apoptosis [53,108]. This raises the possibility that an increase in IL-18 in diabetic nephropathy may proceed the observed increase in IL-6. IL-18 is constitutively expressed in renal tubular epithelia, and infiltrating monocytes, macrophages and proximal tubular cells have all been identified as potential sources of IL-18 production [53,113]. Expression of IL-18 is increased in renal biopsies from patients with diabetic nephropathy in proximal and epithelial tubular cells [114] and patients with type 2 diabetes have significantly higher serum and urinary levels of IL-18 compared to healthy controls [108,109,115,116]. Moreover, there is a positive correlation between IL-18 levels in diabetic patients and the development of urinary albumin excretion, with the highest IL-18 levels found in patients with microalbuminuria and clinical albuminuria [108,109,116,117].

Oxidative Stress Stimulates IL Production

Despite increasing evidence supporting a role for ILs in diabetic nephropathy, little is known regarding the stimulus for increased IL production; however, there is evidence to support a role for oxidative stress. Hyperglycemia-induced oxidative stress and advanced glycation end products have been suggested to induce inflammatory cytokines. In healthy volunteers with either normal or impaired glucose tolerance, acute hyperglycemia increases plasma IL-6 and IL-18 concentrations which are blocked by infusion of the antioxidant glutathione 5 min prior to the glucose infusion, indicating that oxidative stress mediates the increase in IL production [111]. In addition, in older diabetic patients with poor glycemic control and asymptomatic individuals with abnormal fasting glycemia, thiobarbituric acid-reactive substances a marker of oxidative stress, and IL-6 levels are independently correlated with C-reactive protein, suggesting that oxidative stress promotes a state of low-grade systemic inflammation in elderly patients with type II diabetes [118,119]. In hyperglycemia, increased oxidative stress results in an increase in the formation and deposition of advanced glycation end products and their receptors (RAGE) in tissues and RAGE stimulation induces the activation of NFκB which may lead to an increase in IL production [120,121]. Also, as noted above, oxidative stress can directly activate the transcription factors NFκB and AP-1 [55,56] leading to the transcription of ILs either directly or through the induction of other cytokines. In support of this scheme, increased IL-1 production from macrophages incubated with glomerular basement proteins isolated from streptozotocin-induced diabetic male rats was found to be advanced glycation end products-dependent [103].

There is also evidence suggesting that IL production is increased in response to increased oxidative stress via the stimulation of alternative pathways in diabetes. In diabetic nephropathy patients, angiotensin receptor blockers suppress oxidative stress and inflammation and provide protection against the progression of diabetic nephropathy [122]. In agreement with this finding, treatment of streptozotocin-induced diabetic male Sprague-Dawley rats with the ACE inhibitor enalapril prevents enhanced IL-6 expression, leading to a decrease in urinary cytokine excretion and a reduction in albuminuria [105]. Angiotensin converting enzyme inhibitors have been shown to decrease levels of oxidative stress in various experimental models. Alternatively, treatment of human renal proximal tubular epithelial cells with TGF-β increases IL-18 mRNA expression [114], and TGF-β is known to be increased by oxidative stress. These data suggest that elevated oxidative stress during diabetes is a potential mediator for increased ILs production (Figure 2).

Tumor Necrosis Factor-alpha (TNF-α)

TNF-α is a pleiotropic cytokine produced mainly in macrophages and monocytes and is involved in systemic inflammation [123,124]. TNF-α induces a local inflammatory response by initiating a cascade of cytokines and increasing vascular permeability, thereby recruiting macrophage and neutrophils to a site of infection [123,124]. TNF-α activates NFκB signaling mediating the transcription of various cytokines involved in cell survival and proliferation, inflammatory responses and cell adhesion, and antiapoptotic factors [125–129]. Because TNF-α is cytotoxic to glomerular, mesangial, and epithelial cells and can induce renal damage [130], it has been shown to play a pathophysiological role in several experimental models of renal disease including lupus nephritis, crescentic glomerulonephritis, mesangial proliferative glomerulonephritis, diabetes, hypertension, and the remnant kidney model of nephropathy [130–133].

A role for TNF-α in diabetic nephropathy is supported by the finding that urinary albumin excretion significantly correlates with renal TNF-α levels and urinary TNF-α excretion in streptozotocin-induced diabetic rats [134,135]. Moreover, the increase in renal TNF-α levels and excretion precede the increase in albuminuria in diabetes. Urinary TNF-α levels are also elevated in type 2 diabetic patients and TNF-α levels rise as diabetic nephropathy progresses, suggesting that increased TNF-α levels contribute to the development of renal damage [135,136]. TNF-α also contributes to sodium retention and renal hypertrophy, which are early characteristic signs of streptozotocin-induced diabetic nephropathy [137]. Renal TNF-α expression, particularly in the glomerulus and tubulointerestitium, is increased in streptozotocin diabetic rat kidneys, and serum TNF-α is increased in type 2 diabetic patients [134,136]. Therefore, TNF-α plays an important role in the incidence and progression of diabetic nephropathy and renal TNF-α levels correlate with markers of diabetic nephropathy.

The relationship between oxidative stress and TNF-α is complex. TNF-α has been shown to increase ROS and ROS have been shown to increase TNF-α levels [138–140]. In the streptozotocin diabetic rat kidney, elevations in TNF-α increase oxidative stress leading to increased albumin permeability and urinary albumin excretion, a common marker of renal injury [140]. Additionally, elevated peroxynitrite levels are associated with increased TNF-α levels and increased glomerular lesion in streptozotocin diabetic rats [15]. These data suggest that TNF-α is upstream of oxidative stress in diabetic nephropathy. In contrast, administration of an SOD mimetic reduces renal TNF-α levels and albuminuria in type 2 diabetic Zucker rats [141], and the antioxidant tocotrienol offers reno-protection to streptozotocin diabetic rats via decreasing oxidative stress and modulating TNF-α and TGF-β–induced inflammation [140]. These studies suggest that oxidative stress is

upstream of TNF-α activation in diabetic nephropathy (Figure 2). Thus, it is difficult to separate oxidative stress and TNF-α in diabetes. Further experimental and clinical studies using antioxidants and/or TNF-α inhibitors are required to determine the relationship between oxidative stress and TNF-α in diabetic nephropathy.

Monocyte Chemoattractant Protein-1 (MCP-1)

Increasing evidences suggest that recruitment of inflammatory cells from the circulation into renal tissue plays a pivotal role in the progression of diabetic nephropathy. In particular, infiltration of activated T cells and monocytes initiate renal damage and eventually lead to a progressive loss of renal function. Chemokine (C-C motif) ligand 2 (CCL2) is a small cytokine belonging to the CC chemotactic chemokine family that is also known as monocyte chemotactic protein-1 (MCP-1) [142–144]. MCP-1 recruits monocytes, memory T cells, and dendritic cells to sites of tissue injury and infection. MCP-1 is expressed by monocytes, vascular endothelial cells, smooth muscle cells, glomerular mesangial cell, and osteoblastic cells. Many cytokines have been shown to stimulate the production of MCP-1 including IL-1, TNF-α, and TGF-β [142–145].

Studies suggest that MCP-1 plays a role in the progression of diabetic renal injury. Cultured mesangial cells, podocytes, and renal tubular epithelial cells produce MCP-1 in the presence of high glucose and advanced glycation end products [146–149]. In streptozotocin-induced diabetes, MCP-1 is upregulated in glomeruli and tubulointerstitium and this increase contributes to renal fibrosis [150,151]. MCP-1-mediated macrophage infiltration contributes to the progression of diabetic nephropathy [95,146], as evidenced by the ability of MCP-1 inhibition to ameliorate the development of diabetic nephropathy in diabetic mice [146,152]. Clinically, urinary MCP-1 excretion and MCP-1 levels in renal biopsies are elevated in diabetic patients [153,154]. Furthermore, elevated renal MCP-1 in diabetic patients is associated with macrophage recruitment, albuminuria, tubulointerstitial injury, and the progression of diabetic nephropathy [152–155]. Elevated MCP-1 excretion in diabetic patients correlates with macroalbuminuria and was prognostic for deterioration of kidney function [93].

The relationship between oxidative stress and MCP-1 in diabetic nephropathy is unclear and requires further elucidation. However, in experimental animals, elevations in oxidative stress increase macrophage recruitment and renal ICAM-1 and MCP-1 expression in type 1 diabetic rats [156]. In addition, mesangial cells from streptozotocin-induced diabetic mice exhibit an increase in oxidative stress and inflammatory cytokines including MCP-1, and antioxidant treatment reduces MCP-1 levels [17]. Clinically, oxidative stress and plasma MCP-1 are significantly elevated in type 1 diabetic patients with microalbuminuria and poor glycemic control when compared with healthy control subjects [157] and increased oxidative stress is associated with elevations in MCP-1 expression in circulating monocytes in type 1 diabetic patients [158]. Plasma MCP-1 is also positively correlated with plasma malondialdehyde (MDA), a marker of oxidative stress, and albuminuria and vitamin E treatment reduced plasma MCP-1 and albuminuria in type 1 diabetic patients [157,158]. Collectively, these data suggest that oxidative stress-induced MCP-1 expression may contribute to the progression of diabetic nephropathy (Figure 2).

Cell Adhesion Molecules (CAMs)

Vascular cell adhesion molecule-1 (VCAM-1), also known as CD106, is expressed on both large and small vessels following endothelial cell stimulation by cytokines such as TNF-α and IL-1 [159–161]. VCAM-1 promotes the adhesion of lymphocytes, monocytes, eosinophils, and basophils to the vascular endothelium. VCAM-1 also functions in leukocyte-endothelial cell signal transduction, and has been implicated in the development of cardiovascular diseases including diabetes [159–161]. Endothelial VCAM-1 expression increased in spontaneously diabetic KKAy mice [161]. Clinically, circulating VCAM-1 levels are elevated in patients with diabetic nephropathy [162] and diabetic patients with albuminuria have high plasma VCAM-1 levels and an increased risk of end-stage renal disease and death [163].

Inter-cellular adhesion molecule-1 (ICAM-1), also known as CD54, is a member of the immunoglobulin superfamily, which includes antibodies and T-cell receptors [164–167]. ICAM-1 is a ligand for β_2-integrins, a receptor found on leukocytes. When activated, ICAM-1 binds β_2-integrins on leukocyte cell surfaces promoting leukocyte adhesion to the endothelium and transmigration [164–167]. Like VCAM-1, ICAM-1 can be expressed by vascular endothelium, macrophages, and lymphocytes and can also be induced by cytokines such as IL-1 and TNF-α [164–168]. NFκB also increases gene transcription of ICAM-1 [164–166].

Hyperglcemia upregulates endothelial ICAM-1 expression [169] and a direct association between renal ICAM-1 expression and progressive renal injury has been shown in experimental animal models of renal diseases including diabetic nephropathy [166–171]. In streptozotocin-induced diabetic rats, anti-ICAM-1 antibody blocks renal macrophage infiltration suggesting that ICAM-1 mediates macrophage infiltration into the diabetic kidney [172]. Furthermore, ICAM-1 deficient "db/db" mice (ICAM-$1^{-/-}$) have a decrease in glomerular macrophage infiltration and are protected from renal injury when compared to ICAM-$1^{+/+}$ "db/db" mice [173], confirming the notion that ICAM-1 is critically involved in diabetic nephropathy. Clinically, macrophage infiltration and ICAM-1 expression are elevated in kidneys of patients with diabetic nephropathy [166] and patients with type 1 and type 2 diabetes have elevated plasma and urinary ICAM-1 concentrations compared to subjects without renal injury [174–176].

There is evidence to support the hypothesis that CAM expression is stimulated by oxidative stress. It is well known that oxidative stress stimulates NFκB-induced CAMs expression. Advanced glycation end product-RAGE interactions also increase ROS formation in mesangial and endothelial cells with subsequent activation of NFκB and the release of inflammatory cytokines including ICAM-1 and VCAM-1 [159]. Diabetic "db/db" mice have elevated albuminuria, oxidative stress, and tubulointerstitial injury together with increased tubuloepithelial ICAM-1 expression [177]. Streptozotocin-induced diabetic rats have higher renal NADPH oxidase expression and urinary lipid peroxidation product

(LPO) in conjunction with increased glomerular ICAM-1 expression [178], and antioxidant treatment with taurine or NADPH oxidase inhibition reduces renal damage and ICAM-1 expression [178,179]. Immunosuppressive treatment with mycophenolate mofetil (MMF) also ameliorates early renal injury via the inhibition of oxidative stress-induced ICAM-1, MCP-1, and TGF-β expression in streptozotocin-induced diabetic rat kidneys [17,156]. These data suggest that oxidative stress is upstream of ICAM-1 activation in the progression of diabetic nephropathy. However, a recent study demonstrated that xanthine oxidase inhibition reduced albuminuria and renal injury in diabetic "db/db" mouse kidneys without a reduction in renal oxidative stress [177]. Instead a reduction in tubular ICAM-1 expression and subsequent reduction in macrophage infiltration and inflammatory cytokines was identified as the mechanisms of reno-protective [177]. It is possible that there were localized decreases in oxidative stress in certain cell types or in other tissue that were not detected in the study.

Perspectives

There is a close association between oxidative stress and inflammation in diabetes and we hypothesize that an increase in oxidative stress-derived inflammation is a major mechanism in the pathogenesis and progression of diabetic nephropathy. In addition, an increase in inflammatory cytokine levels in diabetes may drive a further increase in oxidative stress as renal injury becomes more pronounced setting up a vicious cycle. However, due to the complex and intimate association between increased oxidative stress and increased inflammation, dissecting the temporal nature of the relationship is a very difficult task. We are in the process of establishing an animal model of diabetic nephropathy that better reflects the human condition in which hyperglycemia, hypertension, proteinuria, and a decline in renal function are all present. We are currently studying streptozotocin-induced diabetic spontaneously hypertensive rats (SHR). These rats are hypertensive, hyperglycemic, and albuminuric with reduced renal function. We will use this model of type 1 diabetes to better define what comes first in diabetic nephropathy, oxidative stress or inflammation. A better understanding of the relationship between oxidative stress and inflammatory cytokines in the progression of diabetic nephropathy will facilitate the development of new treatment options and improve current therapeutic strategies.

Conflict of Interest

The authors have no conflict of interest.

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