Diabetic Nephropathy and Transforming Growth Factor-β: Transforming Our View of Glomerulosclerosis and Fibrosis Build-Up

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The manifestations of diabetic nephropathy may be a consequence of the actions of certain cytokines and growth factors. Prominent among these is transforming growth factor β (TGF-β) because it promotes renal cell hypertrophy and stimulates extracellular matrix accumulation, the 2 hallmarks of diabetic renal disease. In tissue culture studies, cellular hypertrophy and matrix production are stimulated by high glucose concentrations in the culture media. High glucose, in turn, appears to act through the TGF-β system because high glucose increases TGF-β expression, and the hypertrophic and matrix-stimulatory effects of high glucose are prevented by anti–TGF-β therapy. In experimental diabetes mellitus, several reports describe overexpression of TGF-β or TGF-β type II receptor in the glomerular and tubulointerstitial compartments. As might be expected, the intrarenal TGF-β system is triggered, evidenced by activity of the downstream Smad signaling pathway. Treatment of diabetic animals with a neutralizing anti–TGF-β antibody prevents the development of mesangial matrix expansion and the progressive decline in renal function. This antibody therapy also reverses the established lesions of diabetic glomerulopathy. Finally, the renal TGF-β system is significantly up-regulated in human diabetic nephropathy. Although the kidney of a nondiabetic subject extracts TGF-β1 from the blood, the kidney of a diabetic patient actually elaborates TGF-β1 protein into the circulation. Along the same line, an increased level of TGF-β in the urine is associated with worse clinical outcomes. In concert with TGF-β, other metabolic mediators such as connective tissue growth factor and reactive oxygen species promote the accumulation of excess matrix. This fibrotic build-up also occurs in the tubulointerstitium, probably as the result of heightened TGF-β activity that stimulates tubular epithelial and interstitial fibroblast cells to overproduce matrix. The data presented here strongly support the consensus that the TGF-β system mediates the renal hypertrophy, glomerulosclerosis, and tubulointerstitial fibrosis of diabetic kidney disease.

THE HISTOLOGY OF THE kidney is altered dramatically in virtually all affected diabetic patients. The early structural changes consist of glomerular and tubuloepithelial hypertrophy. Then, progressive thickening of the glomerular and tubular basement membranes becomes evident over a period of years.[1,2] In those patients destined to develop renal insufficiency, extracellular matrix proteins accumulate in the mesangium, obliterating the surrounding glomerular capillaries and reducing the glomerular filtration rate.[3] In a similar manner, extracellular matrix accumulates in the tubulointerstitium and around the arterioles, contributing to the destruction of individual nephrons.[4] Given the importance of glomerulosclerosis and tubulointerstitial fibrosis in the development and progression of diabetic nephropathy, basic research activity has focused largely on the mechanisms that lead to increased synthesis or decreased degradation of extracellular matrix.

In the past decade, we have learned that one effector molecule primarily is responsible for stimulating renal cells to undergo hypertrophy and to overproduce matrix proteins. These biologic changes are provoked by transforming growth factor β (TGF-β), a hypertrophic and prosclerotic cytokine that affects glomerular cells, tubular cells, and interstitial fibroblasts. TGF-β has been shown to mediate virtually all of the pathologic changes of diabetic kidney disease.[5]

STIMULANTS OF TGF-β IN THE DIABETIC KIDNEY

Many features of the diabetic state stimulate renal TGF-β activity. Hyperglycemia,[6-8] increased nonenzymatic glycation of proteins,[9-11] de novo synthesis of diacylglycerol and subsequent activation of protein kinase C,[12] increased intracellular glucosamine production,[13,14] and enhanced renal production of vasoactive agents such as angiotensin II,[15] endothelins,[16] and thromboxane[17] all have been shown to increase the expression of TGF-β in both cell culture and in vivo systems. Intraglo-

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merular hypertension, modeled in cell culture by the cyclic stretch and relaxation of mesangial cells and increased fluid shear stress on endothelial cells also increase TGF-β production and bioactivity.[18,19]

EFFECTS OF TGF-β ON KIDNEY HYPERTROPHY AND MATRIX EXPRESSION

Once activated, the TGF-β system induces the accumulation of matrix in multiple and cooperative ways. It stimulates the messenger RNA (mRNA) expression and protein production of key extracellular matrix molecules including type I collagen, type IV collagen, fibronectin, and laminin.[20] At the same time, it impedes the degradation of extracellular matrix by inhibiting the production of proteases that digest matrix (eg, plasminogen activator, collagenase, elastase, and stromelysin) and activating the inhibitors of those proteases (eg, tissue inhibitors of metalloproteinases and plasminogen activator inhibitor 1).[21] TGF-β also upregulates integrins, the cell surface receptors for extracellular matrix, thereby enhancing the ability of cells to interact with specific matrix proteins.[22] Additionally, TGF-β has a potent chemotactic property that can attract fibroblasts and other phagocytic cells,[23] and it has a peculiar ability to induce its own expression,[24] potentially amplifying the fibrotic response.

TGF-β also mediates renal cellular hypertrophy, another characteristic of diabetic nephropathy. It interferes with normal regulation of the cell cycle by inducing cyclin-dependent kinase inhibitors such as $p27^{Kip1}$ and $p21^{Cip1}$.[25] These inhibitors also are increased by high glucose and the diabetic state.[26-28] They suppress the activity of cyclin-dependent kinases, predominantly cyclin-dependent kinase 2/cyclin E kinase,[29] thus inhibiting the phosphorylation of retinoblastoma protein and arresting a cell in the late G1 phase. The cell enters a period of protein synthesis without DNA replication and undergoes hypertrophy. Thus, TGF-β causes changes at the cellular level that translate into the pathophysiologic features of diabetic nephropathy.

EVIDENCE FROM CELL CULTURE

High Glucose Effects Predominantly Are Mediated by the TGF-β System

To mimic the effects of diabetes on the kidney, researchers have grown different renal cell types in tissue culture under high ambient glucose conditions. High glucose stimulates proximal tubular[30,31] and mesangial cell hypertrophy,[7,28,32] and it stimulates the production of matrix molecules such as fibronectin and collagens in proximal tubule cells and glomerular mesangial, epithelial, and endothelial cells.[8,30,33-40] Cell culture studies also have shown that renal cortical fibroblasts produce excess type I collagen under high glucose conditions.[41] In rat mesangial cell and human tubulointerstitial cell culture, periodically increased glucose levels increase collagen production to a greater extent than persistently increased glucose concentrations.[42,43] This more closely mimics the fluctuation of blood glucose levels in vivo and may highlight the detrimental effects of labile hyperglycemia on the pathogenesis of diabetic glomerulosclerosis.

In most kidney cell types, high ambient glucose up-regulates the expression and bioactivity of TGF-β, which itself has been shown to mediate the hypertrophic and profibrotic effects of high glucose. Mesangial cells,[8,44] glomerular endothelial cells,[45] proximal tubular cells,[6] and interstitial fibroblasts[41] incubated in high glucose have increased expression of TGF-β1 and in some cases TGF-β type II receptor,[40,46] which directly binds to the TGF-β ligand. This enables TGF-β1 to act in an autocrine or paracrine fashion to effect significant changes in cellular behavior. For example, murine mesangial cells initially show increased proliferation in high glucose, but after 72 hours the cells show decreased proliferation owing to high glucose–induced TGF-β, which has hypertrophic/growth inhibitory effects[7] that may be mediated partially by $p27^{Kip1}$.[28] Even in the absence of high glucose, addition of exogenous TGF-β1 causes the mesangial cells and the interstitial fibroblasts to increase their expression and production of collagen matrix proteins, showing that TGF-β can reproduce the effects of high glucose.[8,41,46] Finally, antagonism of TGF-β by specific neutralizing monoclonal antibodies[47] or by antisense oligonucleotides[48] significantly decreases and even completely abolishes the high glucose–induced increase in extracellular matrix expression, indicating that TGF-β predominantly mediates the profibrotic effect of high glucose on kidney cells.

Certainly, not all of the high glucose effects are mediated by the TGF-β system. High glucose stimulates the expression and production of type IV

collagen by the cultured, differentiated podocyte, but rather than increasing all the α chains of collagen IV, exogenous TGF-β1 actually decreases certain α chains.[40] Specifically, high glucose increases the α1, α3, and α5 chains of collagen IV.[40] On the other hand, exogenous TGF-β1 stimulates α3 but inhibits the expression of α1 and α5(IV) collagen. Although it is unlikely that the high glucose effects on α1 and α5(IV) collagen would be mediated by TGF-β in the podocyte, the high glucose–induced production of α3(IV) collagen is prevented completely by an inhibitor of TGF-β signaling (SB-431542).[40,49] To ascertain the mechanism of this TGF-β–mediated effect on α3(IV) collagen, the effects of high glucose were studied on components of the TGF-β system. Contrary to other renal cell types, the podocyte did not respond to high glucose with a significant increase in TGF-β1 ligand.[40] Rather, it increased its cell surface expression of the TGF-β type II receptor. In this way, high glucose activates the TGF-β system in podocytes, adding a variation to the theme that high glucose stimulates TGF-β activity in renal cells.

Role of Smads in TGF-β Signaling

Moving beyond high glucose to probing the mechanisms of TGF-β signaling, we investigated the role of the Smad pathway, which transduces the TGF-β signal from the receptor complex to the nucleus. Our data suggest that high glucose may exert some of its effects on extracellular matrix expression through the system of intracellular Smad proteins. In mouse mesangial cells, high glucose stimulates the transcription of fibronectin and, furthermore, potentiates the transcriptional activation of fibronectin by TGF-β1.[50] This particular effect of TGF-β1 appears to be mediated by the receptor-activated Smads, which include Smad2 and Smad3. Smad2 was not investigated, but overexpression of Smad3 alone was able to induce fibronectin promoter activity. In conjunction with exogenous TGF-β1, Smad3 overexpression synergistically increased fibronectin expression, as if the extra Smad3 had increased the efficiency of TGF-β signaling. Finally, transfection of a Smad3-dominant-negative construct was able to inhibit TGF-β1 from stimulating the promoter activity of fibronectin.[50] However, part of the TGF-β1–induced fibronectin expression also may be mediated in parallel by the p38 mitogen-activated protein kinase pathway.[49] Finally, there is evidence to suggest that Smad3 predominantly mediates the effect of TGF-β1 to increase the mRNA expression of α1(I) collagen.[49]

TGF-β Cooperates With Hyperglycemia

TGF-β and high glucose also can interact by an insidious mechanism. High glucose increases the activity of TGF-β, but TGF-β in turn can augment the effect of high glucose. In both human and rat mesangial cells, TGF-β has been shown to up-regulate the mRNA expression and protein production of the insulin-independent, transmembrane glucose transporter, GLUT1,[51,52] thus facilitating glucose uptake and increasing the flux of glucose through its biochemical pathways.[53] Intermediates in glucose metabolism can activate signaling pathways such as protein kinase C[54] and the hexosamine pathway[14] that then stimulate the TGF-β system even further. In deciphering the mechanism by which high glucose increases GLUT1, Inoki et al[51] found that the addition of neutralizing anti–TGF-β antibody prevented the stimulatory effects of high glucose on GLUT1 expression. Interestingly, overexpression of GLUT1 protein in cultured rat mesangial cells caused a marked increase in glucose uptake and the synthesis of extracellular matrix molecules, even when grown in normal ambient glucose concentrations.[55,56] Thus, TGF-β and GLUT1 are both up-regulated by a hyperglycemic milieu, and each can influence the expression of the other.

EVIDENCE FROM ANIMAL MODELS

Intrarenal TGF-β Is Increased by Diabetes

In experimental animal models, TGF-β has been shown to play an important role in the pathogenesis of diabetic kidney disease. Several groups of investigators have shown that the TGF-β level is increased in the kidneys of insulin-dependent diabetic animals during both early and late stages of disease.[57-65] A progressive increase in the TGF-β1 mRNA and protein levels was noted in glomeruli isolated from the streptozotocin (STZ)-induced diabetic rat[57,58] in association with an increased expression of extracellular matrix molecules.[66] Treatment of the STZ-diabetic rat with sufficient insulin to reduce hyperglycemia ameliorated the enhanced expression of TGF-β[63] and matrix components in the glomeruli.[57,58]

Increased TGF-β expression in the kidney may manifest very early after the onset of diabetes. In our study on the spontaneously diabetic Biobreeding rat (Bio-Breeding Labs, Ontario, Canada) and the nonobese diabetic mouse, we found increased TGF-β1 mRNA and protein levels in the kidney cortex as early as a few days after the appearance of glycosuria and coincident with the development of renal hypertrophy.[60] In the STZ-diabetic rat and mouse, increased TGF-β1 expression in the renal cortex and glomeruli was noted as early as 1 to 3 days after the onset of diabetes.[63,67] Interestingly, up-regulation of the TGF-β type II receptor mRNA and protein also occurred early in the natural history of STZ-diabetic rodents.[46,65,67]

The intrarenal TGF-β system also is activated in animal models of type 2 diabetes. The *db/db* mouse, characterized by hyperglycemia, obesity, and insulin resistance, develops increased amounts of TGF-β1 that are localized to the glomerular compartments.[68] In contrast, the mRNA and protein levels of the TGF-β type II receptor are significantly up-regulated in both the glomerular[68] and the tubulointerstitial compartments.[68] Overall, the increased glomerular TGF-β and the more widespread increases in TGF-β type II receptor result in activation of the renal TGF-β system and stimulation of the downstream Smad signaling cascade. By immunohistochemistry of the diabetic *db/db* mouse (compared with the *db/m* mouse), Smad3 was found to accumulate in the nuclei of glomerular and tubular cells where Smad proteins could influence the expression of genes that are regulated by TGF-β signaling.[68] More evidence of Smad nuclear translocation could be seen by Southwestern histochemistry in which labeled oligonucleotides comprising the Smad binding element increasingly were localized to the nuclei of glomerular and tubular cells of diabetic mice,[68] suggesting increased transcription of genes that are modulated by TGF-β. Thus, the net bioactivity of the renal TGF-β system is increased in the type 2 diabetic *db/db* mouse.

Intervention With Anti–TGF-β Therapies

The development of diabetic renal hypertrophy and glomerulosclerosis likely is caused by heightened activity of the TGF-β system. Short-term treatment of the STZ-diabetic mouse with a neutralizing monoclonal antibody against all 3 isoforms of TGF-β prevented glomerular hypertrophy, reduced the increment in kidney weight by 50%, and significantly attenuated the increase in TGF-β1, α1(IV) collagen, and fibronectin mRNAs without affecting glycemic control.[67] The results of this study suggested a cause and effect relationship between the renal TGF-β system and the development of early structural changes in diabetic nephropathy.

To expand on these findings, we conducted a similar study, this time on the *db/db* mouse, to examine whether long-term anti–TGF-β antibody treatment would ameliorate the late structural changes and functional consequences of diabetic nephropathy.[69] We found that systemic anti–TGF-β therapy for 8 weeks prevented the mesangial matrix expansion of diabetic glomerulosclerosis and, most importantly, the treatment preserved kidney function, showing that neutralization of TGF-β activity could prevent the progression of renal failure in diabetes. However, the anti–TGF-β antibody did not reduce albuminuria, which itself may promote the progression of renal insufficiency.[70] The paradox of preserved renal function in the face of persistent albuminuria may perhaps be explained by postulating that the deleterious effects of proteinuria are mediated themselves by the TGF-β system.[71]

However, prevention of diabetic nephropathy in humans is not always feasible. More often than not, the physician has to treat diabetic kidney disease that is far advanced, with pathologic lesions that are well established. It used to be thought that the structural damage of diabetic nephropathy was irreversible, so treatment recommendations focused on preventing further injury and slowing the rate of decline in renal function. More recently, however, physicians have contemplated the reality of curing diabetic nephropathy. If diabetes could be treated optimally, then perhaps the kidney could heal itself. We reasoned that if TGF-β mediates most of the renal damage in diabetes, then neutralizing TGF-β overactivity might not only prevent but also reverse the structural lesions of diabetic nephropathy. We performed a study in *db/db* mice similar to the study described earlier with anti–TGF-β antibodies, but instead of starting treatment with the onset of diabetes (preventive trial), we started treatment after the establishment of diabetic kidney disease (therapeutic trial). Compared with the control diabetic mice, the treated *db/db* mice displayed significant improvements in the glomer-

ular basement membrane thickening and in the index of mesangial matrix expansion.[72] These structural parameters approached the normal measurements of the nondiabetic *db/m* mice. Even at this late stage and even though the hyperglycemia was left untreated, antagonizing the intrarenal TGF-β system was able to at least partially reverse the histologic lesions of diabetic glomerulopathy.

Additional Parts of the TGF-β System

In addition to TGF-β1, other members of the TGF-β family deserve mention. Although it is much less studied, TGF-β2 is believed to play a fibrogenic role.[73] Daily injections of human recombinant TGF-β2 to adult mice caused tissue levels of endothelin-1 and angiotensin II to increase in the kidney and fibrosis to develop in the cortical tubulointerstitium and vasculature.[73] TGF-β2 and other TGF-β system components also have been examined in the STZ-induced diabetic rat and the genetically prone Biobreeding rat.[65] Interestingly, although renal TGF-β1 mRNA levels were increased in the first 30 days after STZ induction, the corresponding TGF-β1 protein did not increase. TGF-β2, however, showed the opposite profile. Its mRNA expression did not increase significantly, but its protein content increased by 2-fold after 30 days of diabetes. Finally, TGF-β type II receptor showed a 3-fold increase in protein by day 90 of STZ induction, making this the most responsive of the TGF-β receptor subtypes. Because TGF-β2 seemed to correlate better with fibrogenesis in the diabetic kidney, the same research group used a human monoclonal anti–TGF-β2 antibody to treat STZ-diabetic rats.[74] Compared with nondiabetic controls, the untreated diabetic rats had increased kidney weights, urinary albumin excretion rates, and protein synthesis of collagen I. Therapy with an anti–TGF-β2 antibody, however, prevented diabetes from increasing these measures of disease. The investigators conclude that the anti–TGF-β2 regimen had a renoprotective effect, and they extrapolate from the attenuation of collagen I that targeting TGF-β2 would suppress kidney fibrogenesis in diabetes.[74] Nevertheless, TGF-β1 remains the most abundant and most studied isoform in the kidney. The importance of TGF-β2 or TGF-β3 is not as well established. Future studies will need to address the specific role that each isoform plays in diabetic nephropathy.

EVIDENCE FROM HUMAN STUDIES

Increased TGF-β in Human Diabetic Nephropathy

Studies performed in diabetic patients with various degrees of nephropathy also implicate the renal TGF-β system in the development of human diabetic renal disease. All 3 isoforms of TGF-β have been discovered to be increased in both the glomerular and the tubulointerstitial compartments of patients with established diabetic nephropathy.[58,75,76] Furthermore, glomerular TGF-β1 mRNA, measured by the reverse-transcription polymerase chain reaction method, was increased markedly in renal biopsy specimens from patients with proven diabetic kidney disease.[77] These investigations support the belief that increased renal TGF-β levels correlate closely with the degree of mesangial matrix expansion, interstitial fibrosis, and renal insufficiency.

Another study was designed to determine if diabetic patients have enhanced renal production of TGF-β.[78] Aortic, renal vein, and urinary levels of TGF-β were measured in 14 type 2 diabetic and 11 nondiabetic control patients undergoing elective coronary artery catheterization. Both groups were matched roughly with regard to the range of renal function and the presence of hypertension and proteinuria. Renal blood flow was measured to calculate the net mass balance across the kidney. The gradient of TGF-β1 concentration across the renal vascular bed was negative in the nondiabetic patients, indicating net renal extraction of TGF-β1, whereas the gradient was positive in the diabetic patients, indicating net renal production of TGF-β1. When the renal TGF-β1 mass balance was calculated, a similar pattern was observed, with the nondiabetic kidney removing approximately 3,500 ng/min of TGF-β1 from the circulation, and the diabetic kidney adding approximately 1,000 ng/min of TGF-β1 to the circulation. In addition, the level of bioassayable TGF-β was increased 4-fold in the urine of diabetic versus nondiabetic patients. The increased urinary TGF-β was not simply a function of enhanced glomerular permeability to protein because diabetic patients both with and without microalbuminuria displayed similarly high rates of urinary TGF-β excretion. These results support the conclusion that the kidneys of diabetic patients overproduce TGF-β1 protein. The details of this phenomenon and the exact contribution of

the different renal cell types to TGF-β1 production need to be investigated.

TGF-β Levels Correlate With Outcomes

An interesting post hoc study[79] assessed whether captopril treatment would lower serum TGF-β1 levels in a small subset of patients with diabetic nephropathy who had been enrolled in the Collaborative Study Group.[80] After 6 months, the serum TGF-β1 level decreased significantly by 21% in the captopril-treated group, whereas it increased slightly by 11% in the placebo-treated group. Interestingly, the captopril-treated patients who had a decrease in the serum TGF-β1 level tended to have better preserved renal function over the ensuing 2-year period. This association was even more pronounced in the subset of patients with an initial glomerular filtration rate of less than 75 mL/min. These results suggest that TGF-β1 plays a pivotal role in the progression of diabetic nephropathy and that angiotensin converting enzyme inhibitor therapy may protect the kidney by lowering TGF-β1 production.

More recently, the EURODIAB Prospective Complications Study examined the correlation between levels of TGF-β1, Amadori albumin, and the microvascular complications of type 1 diabetes.[81] An increased level of circulating TGF-β1 was associated with an increased prevalence of proliferative retinopathy. On the other hand, increased urinary TGF-β1 levels were correlated highly with the severity of albuminuria. Both of these parameters were largely accounted for in the multivariate model by the changes in blood pressure, glycemic control, and levels of Amadori albumin. Perhaps these features of the diabetic state, given their impact on urinary TGF-β1 levels, should be aggressively controlled to reduce the risk for progression to microalbuminuria, the incipient stage of diabetic nephropathy.

TGF-β Regulation and Propensity for Diabetic Nephropathy

Factors that regulate the bioavailability of TGF-β also influence the predisposition to diabetic kidney disease. One such factor is the family of latent TGF-β binding proteins (LTBP). These regulatory molecules covalently bind with the small latent forms of TGF-β, facilitating the efficient secretion of TGF-β[82] and targeting the TGF-β complex to the extracellular matrix.[83] The relevance of LTBP to human diabetic nephropathy can be seen in a study that tried to link expression levels of TGF-β components with the likelihood of developing diabetic nephropathy.[84] Type 1 diabetic patients were ranked according to their severity of mesangial expansion and their duration of diabetes and then were categorized into fast-track and slow-track risk groups for the development of diabetic nephropathy. From these 2 cohorts and normal control subjects, skin fibroblasts were cultured in high glucose and then assayed for mRNA levels (by real-time reverse-transcription polymerase chain reaction) of TGF-β1, type II receptor, thrombospondin-1, and LTBP-1. No differences were found in the mRNA expression of TGF-β1, type II receptor, or thrombospondin-1 between fast-track and slow-track patients. The only significant difference between the 2 groups was found with LTBP-1.[84] Slow-track patients had lower levels of LTBP-1 than normal or fast-track patients, suggesting that the decreased LTBP-1 and presumably the decreased TGF-β bioavailability may have protected the slow-track patients from developing diabetic nephropathy as quickly. Therefore, with regard to TGF-β regulation, genetic variability of LTBP levels seems to play an important role in the susceptibility to diabetic renal disease.

OTHER FACTORS THAT INTERACT WITH THE TGF-β SYSTEM

Another growth factor has been discovered to act downstream of TGF-β. Named *connective tissue growth factor* (CTGF), this prosclerotic cytokine is one of the TGF-β–inducible immediate early genes[85] and is induced in cultured mesangial cells by TGF-β.[86] The transcriptional mechanism by which TGF-β induces CTGF gene expression involves the Smad binding elements and a unique TGF-β response element in the CTGF promoter.[87,88] Consistent with the paradigm that CTGF works downstream of TGF-β, in vitro studies of renal cells, including mesangial cells, indicate that CTGF mediates TGF-β–stimulated matrix protein expression. For example, CTGF has been shown to mediate TGF-β–induced increases in fibronectin[89,90] and collagen type I.[91] Furthermore, in mesangial cells, hyperglycemia induces CTGF by mechanisms that depend partly on the TGF-β system and partly on the protein kinase C pathway.[92,93] In animal studies, the expression of

CTGF was found to be increased in experimental diabetic glomerulosclerosis.[94] Increased CTGF levels in the glomeruli of nonobese diabetic mice appear to correlate with the duration of diabetes.[89] In the *db/db* mice, quantitative reverse-transcription polymerase chain reaction showed that glomerular CTGF transcripts increased by an impressive 27-fold after 3.5 months of diabetes. The changes in CTGF expression occurred early in the course of mesangial matrix expansion, interstitial disease, and proteinuria, implicating an important role for CTGF in the development of diabetic glomerulosclerosis.[95]

The link between oxidative stress and TGF-β is gaining increasing attention in mediating diabetic renal injury. Oxidative stress, generated by glucose metabolism and advanced glycation end-products, can trigger a multitude of pathogenetic mechanisms that contribute collectively to the microvascular complications of diabetes.[96] A major component of oxidative stress, the reactive oxygen species, may act through the TGF-β pathway to exert a profibrotic effect. To generate reactive oxygen species under experimental conditions, investigators have used glucose oxidase, an enzyme that continuously catalyzes ambient glucose to hydrogen peroxide. The addition of glucose oxidase to human mesangial cells in culture stimulates the promoter activity, mRNA level, bioactivity, and protein production of TGF-β1.[97] Glucose oxidase also increases the gene expression of several extracellular matrix proteins including collagen types I, III, and IV, and fibronectin. However, this glucose oxidase–stimulated expression of matrix was prevented by a panselective, neutralizing, anti–TGF-β antibody.[97] Thus, the reactive oxygen species may exert their deleterious effects on kidney cells via the TGF-β system.

Oxidative stress also has been shown to activate the protein kinase C pathway. Recent data have shown that inhibition of high glucose–induced protein kinase C activation effectively abrogates reactive oxygen species generation and nuclear factor κ B activity, decreasing monocyte chemoattractant protein-1 secretion in mesangial cells.[98] Transcription factors such as nuclear factor κ B enhance the transactivation of genes encoding cytokines such as TGF-β and CTGF that up-regulate extracellular matrix expression.[97,99] Taken together, evidence is accumulating to suggest that the different biochemical abnormalities produced by hyperglycemia can influence one another because many of the glucose metabolites serve as important intermediates for the different metabolic pathways.

TUBULOINTERSTITIUM IN DIABETIC NEPHROPATHY

Shifting the focus away from the glomerulus, the tubulointerstitium also plays an important role in the progression of diabetic nephropathy.[4,100] The extent of tubulointerstitial fibrosis correlates best with the rate of deterioration in glomerular filtration rate in all kidney diseases including diabetic nephropathy.[101,102] Recent studies have implicated an important role for TGF-β in the development of tubulointerstitial fibrosis. Interstitial fibroblasts from normal mice react to high ambient glucose by increasing the synthesis of TGF-β.[41] As a result, fibroblasts proliferate and increase their production of type I collagen.[41] Exaggerated production of TGF-β1 also was seen with proximal tubular cells cultured in high levels of albumin, a scenario that corresponds with the progression to diabetic proteinuria.[103] This observation may help to elucidate the pathophysiology behind the toxic effects of excessive protein ultrafiltration in glomerular diseases.[104] Proximal tubular cells exposed to protein overload acquire an inflammatory and profibrotic phenotype[105] that results in the increased generation of TGF-β1.[103] Other studies have confirmed these findings in cultured human tubulointerstitial cells and also reported exaggerated fibrogenic responses to intermittent exposures to high glucose.[43] As with previously mentioned studies in mesangial cells,[42] this simulated labile hyperglycemia may be more detrimental for the development of interstitial fibrosis.[43] Furthermore, it has been hypothesized that in disease states characterized by tubulointerstitial fibrosis, resident tubular epithelial cells may give rise to interstitial fibroblasts by epithelial-mesenchymal transformation.[106-108] This process is strongly evoked by TGF-β and other profibrotic factors such as fibroblast growth factor-2.[109] Interestingly, TGF-β has been detected in myofibroblasts within the interstitial space of kidneys affected by diabetes but not in normal kidneys.[110] This TGF-β/myofibroblast axis is believed to overproduce extracellular matrix, perhaps contributing to tubulointerstitial fibrosis in diabetes.[110]

CLOSING THOUGHTS AND FUTURE APPLICATIONS

Any discussion on the pathophysiology of diabetic nephropathy must acknowledge the role of the renin-angiotensin system (RAS). Innumerable clinical trials have established that angiotensin-converting enzyme (ACE) inhibitors delay the progression of diabetic kidney disease.[80,111,112] More recently, the class of angiotensin receptor blockers (ARBs) has been shown to slow the loss of renal function in type 2 diabetic nephropathy.[113-115] The clinical benefit of renin-angiotensin blockade classically has been attributed to the relaxation of efferent arteriolar constriction and the release of intraglomerular pressure, but this view has been expanded to consider the nonhemodynamic mechanisms of renal injury by angiotensin acting as a cytokine.[15,116] Angiotensin II normally stimulates the biosynthesis of matrix by cultured renal cells,[117-119] so the ACE inhibitors or ARBs might be expected to inhibit matrix formation and thereby ameliorate the sclerosis of diabetic nephropathy. Further, the action of angiotensin II on matrix production appears to be mediated by the renal cellular TGF-β system because angiotensin II stimulates TGF-β1 expression, and various anti–TGF-β regimens successfully have abolished the angiotensin II–induced increases in collagen I, collagen IV, and fibronectin.[119-122] Thus, the antifibrotic and renoprotective effects of angiotensin blockade are related partly to its ability to reduce TGF-β overexpression in the kidney. Indeed, ACE inhibitors or ARBs decrease the intrarenal levels of TGF-β1, both in animal models of diabetes and in human diabetes.[123-127]

However, neither ACE inhibitors nor ARBs have been able to provide complete renoprotection. Despite optimal treatment, some diabetic patients still progress to end-stage renal disease. To achieve more effective blockade of the RAS, physicians have started to combine ACE inhibitors with ARBs, rationalizing that the combination would protect the kidney from diabetic injury better than either medication alone.[128] Thus far, the hypothesis has been upheld by the available clinical trials in that the combinations lower blood pressure and albuminuria/proteinuria to a greater degree.[129-131] Addition of an ARB to maximal ACE inhibitor therapy also was able to suppress urinary TGF-β1 levels even further than the ACE inhibitor, suggesting that more comprehensive blockade of the RAS would confer extra renoprotection.[132] Nevertheless, ACE inhibitors plus ARBs do not totally normalize the levels of TGF-β in the diabetic kidney.[133,134] The failure to correct TGF-β overactivity may explain why the ACE inhibitor/ARB combinations may not prevent or even delay end-stage renal disease in every patient. Suggestive of this, one trial has found that an ARB added to an ACE inhibitor did not improve diabetic proteinuria.[135] Whether the ACE inhibitors together with ARBs can preclude diabetic kidney failure remains to be seen, but at least in chronic nondiabetic renal disease, dual blockade with trandolapril and losartan did not entirely prevent the need for renal replacement therapy.[136]

These observations reinforce the multifactorial nature of diabetic nephropathy and suggest that in addition to the RAS, other pathogenetic routes must be blocked by targeted therapies in a coordinated attack on diabetic kidney disease. We propose that on top of the tried-and-true treatments for diabetic nephropathy (eg, tight glycemic control and strict blood pressure control, preferably with ACE inhibitors and/or ARBs), anti–TGF-β therapies should be added, with the goal of suppressing renal TGF-β activity back to normal. Given the importance of the TGF-β system in the pathophysiology of diabetic renal disease and its interplay with the RAS, future methods that intercept the renal TGF-β axis to arrest the damaging effects of fibrosis likely will complement the mainstays of therapy. Clinical trials of specific anti–TGF-β regimens should be feasible in humans and may one day become an important facet of a multipronged approach to diabetes and its complications.

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