Perspectives in Diabetes

Hyperglycemia and Diabetic Kidney Disease

The Case for Transforming Growth Factor–β as a Key Mediator

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Renal cells are a rich source of transforming growth factor (TGF)-β, and they serve as targets for its actions. Our hypothesis that activation of the TGF-β system in the kidney is implicated in the development of diabetic renal disease stems from the close similarity of actions of TGF-β and high ambient glucose on renal cell growth and extracellular matrix metabolism. Proximal tubule cells and glomerular mesangial cells cultured in high glucose concentration express increased TGF-β1 mRNA and protein levels, and treatment with anti-TGF-β antibodies results in prevention of the effects of high glucose to induce cellular hypertrophy and stimulate collagen biosynthesis. Several in vivo studies by different groups of investigators have reported overexpression of TGF-β in the glomeruli in human and experimental diabetes. We have also observed that the development of renal hypertrophy in the insulin-dependent diabetic BB rat and NOD mouse is associated with increased expression of TGF-β1 in the kidney and that short-term administration of antibodies capable of neutralizing the activity of TGF-β in the streptozotocin mouse model of diabetes results in attenuation of whole kidney and glomerular hypertrophy and overexpression of mRNAs encoding matrix components. Together, these findings are consistent with the hypothesis that the diabetic state stimulates TGF-β expression in the kidney and that in turn this growth factor may mediate, in an autocrine/paracrine manner, some of the principal early manifestations of diabetic renal disease. Demonstrating a causal link between upregulation of glomerular TGF-β and the subsequent development of diabetic glomerulosclerosis will require long-term interventional studies designed to intercept the TGF-β system in the kidney. Diabetes 44:1139–1146, 1995

Renal complications of diabetes are a major cause of morbidity and mortality, but the basic mechanisms for their development remain elusive. The Diabetes Control and Complications Trial has demonstrated that the degree of hyperglycemia is an important predictor of diabetic renal complications, suggesting that the metabolic consequences of hyperglycemia may induce renal pathology (1). In the population with overt diabetic nephropathy, treatment with captopril, an angiotensin-converting enzyme inhibitor (ACEI), significantly delays the progression to end-stage renal failure, suggesting that glomerular hemodynamics and the local renin-angiotensin system are playing an important role (2). However, it is apparent that tight control of blood glucose concentration is not possible for many diabetic patients and that although treatment with an ACEI may delay disease, it does not arrest its progression. Clearly, a mechanistic understanding of the basic processes underlying the development of diabetic nephropathy would help direct future therapies.

Diabetic nephropathy is characterized by hypertrophy of both glomerular and tubular elements, thickening of the glomerular and tubular basement membranes, progressive accumulation of extracellular matrix components in the glomerular mesangium, and tubulointerstitial fibrosis (3–5). Investigations in glomerular mesangial cells and proximal tubule cells have demonstrated that an elevated glucose concentration in the culture medium stimulates the biosynthesis of collagen and other extracellular matrix constituents (5) and modulates the growth of the cells, including the development of tubular epithelial hypertrophy (6,7). In general, these effects of high glucose concentration may arise as a consequence of increased de novo synthesis of diacylglycerol and activation of protein kinase C (PKC) (8,9), early or advanced nonenzymatic glycation (10,11), increased activity of the polyol pathway and disordered myo-inositol metabolism (12), or enhanced synthesis and/or response to hormones, cytokines, or growth factors. Our previous studies were the first to implicate one such growth factor, transforming growth factor (TGF)-β, as an important mediator of the high glucose-induced effects on renal tubular and mesangial cell growth and collagen biosynthesis (13–15). In this review, we will discuss several basic aspects of TGF-β action and
then summarize the evidence implicating TGF-β in the pathogenesis of diabetic kidney disease.

**TGF-β AND ITS RECEPORS**

TGF-β exists commonly as a homodimer bound to its latency-associated peptide. It may be in a soluble form or bound to extracellular matrix molecules (16). When associated with other proteins, it is often in its latent form and thus has poor affinity to bind to its specific receptors on the cell membrane. Activation of latent TGF-β in vivo may largely be controlled by the action of plasmin to cleave the latency-associated peptide from the active TGF-β dimer (17). There are at least three mammalian isoforms of TGF-β (β1, β2, and β3). Although the three isoforms appear to be developmentally regulated and to have differential tissue distribution, discrete functions specifically related to each TGF-β isoform have not been clearly identified (18). In the kidney, TGF-β is the most highly expressed, being present mainly in tubular epithelial cells and to a lesser extent in the glomerulus (19). TGF-β3 follows a similar pattern of expression but in lesser amounts. TGF-β2 is restricted mostly to the juxtaglomerular apparatus, co-localizing with renin staining (20). Of the different isoforms, TGF-β1 has been the best characterized and will be referred to in this review as TGF-β unless indicated otherwise.

Virtually every cell type has been described to produce TGF-β and possess receptors for it. Different types of receptors have been identified. Whether the divergent actions of TGF-β that have been previously reported in different cell lines are the consequence of selectivity of the different receptors remains to be firmly established. The type I and type II receptors are unique in that each has a serine-threonine kinase motif in its cytoplasmic domain (21). The type I and type II receptors bind TGF-β1 with a 10-fold greater affinity than TGF-β2 and appear to be ubiquitous. The type III receptor (betaglycan) has a very short cytoplasmic domain that lacks intrinsic signal transduction activity but appears to function mostly to enhance the affinity of the different TGF-β isoforms to the type II receptor (22). Soluble betaglycan, on the other hand, may bind TGF-β and inhibit its presentation to target sites (22). The significance of the recently described type IV (23) and type V receptors (24) is unclear, although the type V receptor has serine-threonine kinase activity (24).

Activation of the cellular pathways that mediate the actions of TGF-β requires as a first step the interaction of the ligand with the type II receptor, which exists as a dimer at the cell surface (25). The type I receptor is able to recognize and bind to the TGF-β–type II receptor complex. Phosphorylation of the type I receptor by the type II receptor is necessary for signal transduction (25). Downstream second-messenger pathways in the cytosol have yet to be clearly elucidated, but they eventually lead to modulatory events in the nucleus such as the stimulation of transcription of matrix molecules including type I collagen (26,27) and fibronectin (28,29). Stimulation of the transcription factor NF-1 by TGF-β appears to underlie stimulation of the type I collagen promoter (26). Novel transcription factors with leucine zipper or zinc finger motifs have been described in cells stimulated with TGF-β (30,31). Other important nuclear events that are intimately involved in the control of cell growth include the inhibition of cyclin D–dependent protein kinase (cdk4) in the early-to-mid G1 phase of the cell cycle (32). This early downregulation of cdk4 prevents the subsequent activation of cyclin E–dependent protein kinase (cdk2); in turn, this leads to prevention of phosphorylation of Rb, the retinoblastoma susceptibility gene product that also acts as a tumor suppressor (32). The differential state of phosphorylation of Rb is critical in regulating DNA synthesis and cell division (32,33). Rb impedes progression through the cell cycle in the late portion of G1, unless it becomes phosphorylated in middle G1 (34). Rb can be phosphorylated by cdk2 and cdk1 (32). TGF-β prevents Rb phosphorylation in cells whose growth is inhibited by TGF-β (35). Other evidence implicates Rb in the shutdown of the c-myc protooncogene by TGF-β; in this case, cells become deprived of c-myc protein, which is required for the completion of G1 (36).

**FUNCTIONS OF TGF-β**

TGF-β is a prototypical multifunctional cytokine, growth being only one of its many functions. TGF-β turns off the inflammatory response by inhibiting T- and B-cell proliferation (37,38). Gene-knockout transgenic mice in which the gene locus for TGF-β1 is selectively deleted (39,40) manifest an autoimmune-like disorder with massive infiltration of lymphocytes and macrophages in many organs such as lungs and heart, but not the renal parenchyma. High doses of recombinant human TGF-β1 administered to normal rats and rabbits result in excess fibrosis in many organ sites, including the liver and kidney (41), thus emphasizing the prosclerotic potential of this cytokine. Isaka et al. (42) have recently introduced plasmid DNA containing cDNA for TGF-β1 or platelet-derived growth factor (PDGF) into rat kidney via the left renal artery and demonstrated that selective glomerular overexpression of TGF-β1 in vivo promotes glomerulosclerosis within 1 week.

Other studies in cell culture systems (43) indicate that TGF-β plays a crucial role in regulating cell proliferation and extracellular matrix production. In the kidney, TGF-β promotes tubulopelithelial cell hypertrophy and regulates the glomerular production of almost every known molecule of the extracellular matrix, including collagens, fibronectin, tenasin, and proteoglycans (43). In addition, TGF-β enhances production of the integrins, which are the cell-surface receptors for matrix molecules (44), and this property further promotes matrix accumulation. TGF-β also blocks the destruction of newly synthesized extracellular matrix by upregulating the synthesis of protease inhibitors, such as plasminogen activator inhibitors, and by downregulating the synthesis of matrix-degrading proteases, such as stromelysin and collagenase (43,45). The ability of TGF-β to enhance matrix synthesis underlies its important role in healing skin wounds and injuries to heart and brain induced by ischemia (46). In fact, topical application of TGF-β improves healing of skin ulcers in rats given large doses of corticosteroids (47). On the other hand, excess production of the cytokine may cause irreversible tissue fibrosis in a host of disease states (48), as will be discussed below for diabetic renal disease.

The phenomenon of upregulation of matrix production in cultured renal cells exposed to TGF-β and the seminal findings implicating glomerular TGF-β in the untoward development of enhanced matrix deposition in a model of diabetes in the renal disease.
self-limited glomerulonephritis in the rat (49,50) have both supported the current view that persistent upregulation of TGF-β in inflammatory or noninflammatory kidney diseases may lead to overproduction of matrix components in the tubulointerstitial and glomerular mesangial compartments (43). These lesions can severely compromise glomerular filtration. In mesangial cells, TGF-β increases the production of type I and IV collagens, fibronectin, and proteoglycans (15,51–53); in glomerular epithelial cells, it increases fibronectin, biglycan, decorin, and type IV collagen (54); in cultured proximal tubular epithelial cells, it enhances the synthesis of type IV collagen (F.N.Z., unpublished observations) and proteoglycans (55); and in cultured renal fibroblasts, it increases production of type I, III, and V collagens (56). In addition, TGF-β is generally hypertrophic to renal cells, stimulating the synthesis of cellular proteins in proximal tubular and mesangial cells as measured by amino acid incorporation (18,57,58). Inhibition of cellular proliferation is another characteristic effect of TGF-β on renal cells such as proximal tubular epithelial cells, glomerular epithelial cells, and glomerular endothelial cells (43,51,59). However, in certain mesenchymally derived cell types (e.g., mesangial cells, smooth muscle cells, osteoblasts, and fibroblasts), TGF-β acts as a bifunctional regulator of growth; it may either stimulate or inhibit proliferation depending on cell culture conditions, TGF-β concentration, or secondary activation of counterregulatory cytokine systems (60,61). For example, if rat mesangial cells are exposed to exogenous TGF-β for several days, they proliferate because of upregulation of PDGF and/or its receptor(s) (60,62). Renal fibroblasts (NRK strain) demonstrate inhibition of cell proliferation in response to TGF-β alone, but they respond by stimulation when TGF-β and epidermal growth factor (EGF) are added together (63).

**ROLE OF TGF-β IN DIABETIC RENAL DISEASE**

Increased protein synthesis and inhibition of cell proliferation by TGF-β indicate that this growth factor stimulates cell hypertrophy. In fact, vascular smooth muscle cells (64), cardiac myocytes (65), proximal tubular epithelial cells (57), and glomerular mesangial cells (58) all exhibit hypertrophy in response to addition of TGF-β to the culture media. The actions of TGF-β on renal cells to induce hypertrophy and stimulate extracellular matrix production, features that are characteristic of diabetic kidney disease, also predict that this growth factor may be involved in the pathogenesis of the disease. This hypothesis (13,66) is supported by the close similarity of actions of TGF-β and high ambient glucose on the growth and extracellular matrix metabolism of renal cells (6). Criteria to establish a role for a cytokine in mediating a specific disease have been adapted from Koch's postulates (67): 1) that the cytokine has a relevant effect on target cells in vitro, 2) that it is produced or released in the disease, 3) that administration of the cytokine in vivo or its overexpression using transient transfection experiments or transgenic animals will reproduce the biological effects, and 4) that inhibition of the cytokine in vivo with neutralizing antibodies or by other means will block the manifestations of the disease. As will be discussed, these criteria have all been fulfilled in diabetic kidney disease, thus establishing a causal relationship between elevated production of TGF-β and disease manifestations (68,69).

**TGF-β mediates effects of high glucose on renal cells**

**In vitro studies.** Cell culture experiments have revealed that the effects of high glucose on renal proximal tubular cells and glomerular mesangial cells are mediated by autocrine production and activation of TGF-β. In both cell types, expression of TGF-β1 mRNA and bioactivity are significantly increased within 48 h of exposure to high glucose concentration in the growth media (13–15,70). Inhibiting TGF-β activity with specific neutralizing antibodies attenuates the glucose-induced inhibition of cell proliferation and the stimulation of protein synthesis, including that of collagen types I and IV (13–15). Furthermore, rat mesangial cells grown in high glucose concentration express decreased collagenase activity, and this action is mediated by autocrine TGF-β (71). TGF-β may also serve a role in enhancing glucose uptake in fibroblasts. When present with serum, TGF-β upregulates GLUT1 mRNA levels and enhances glucose uptake (72). Increased glucose uptake has recently been shown to be a critical feature in mediating matrix synthesis in mesangial cells (73). Together these studies indicate that there exists a reciprocal relationship between TGF-β and glucose whereby high glucose stimulates TGF-β1 production, which in turn enhances glucose uptake, and that both TGF-β and high glucose can cooperate to stimulate matrix accumulation.

**TGF-β mediates diabetic kidney disease**

**In vivo studies.** Several studies by many groups of investigators have demonstrated that TGF-β expression is elevated in the kidneys of animals with insulin-dependent diabetes (74–80). A progressive increase in TGF-β1 mRNA and protein levels was noted in glomeruli isolated from the streptozotocin (STZ)-induced diabetic rat model (74,75) in association with increased expression of matrix molecules (81). Treatment of the rats with sufficient insulin to reduce the hyperglycemia ameliorated the enhanced expression of TGF-β and matrix components in glomeruli (74,75,81). Increased TGF-β expression in the kidney can be manifest very early after the onset of diabetes. In STZ-induced diabetic rats and mice, increased TGF-β1 expression in the renal cortex was noted as early as after 2–3 days of diabetes (69,80). In our study on the spontaneously diabetic BB rat and the NOD mouse, we found increased TGF-β1 mRNA and protein levels in the kidney cortex as early as few days after the appearance of glycosuria and coincident with the development of renal hypertrophy (77). Another group reported an increase in TGF-β1 expression in the glomerulus of NOD mice by 4 weeks of diabetes in association with glomerular hypertrophy (78). Increased renal TGF-β2 (and not TGF-β1) mRNA has also been observed after 5 or 10 days of glycosuria in NOD mice (79). Immunohistochemical examination localized TGF-β2 expression to glomeruli and the interstitium in this study (79). The reason for the discrepancy in the TGF-β isoform among these studies (77–79) is not readily discernible. Serum TGF-β1 levels measured by a bioassay were found to be elevated in STZ-induced diabetic rats (76). In our study on STZ-induced diabetic mice, we found significantly increased urinary, but not serum, levels of total TGF-β1 (active + latent) as measured by enzyme-linked immunosorbent assay (69).

The source of enhanced TGF-β in diabetic kidneys shortly after the onset of diabetes is probably caused by enhanced production of TGF-β by resident renal cells, although a minor contribution by infiltrating cells such as macrophages is also possible. Enhanced intracellular TGF-β staining,
primarily in tubular epithelial cells, has been reported in the spontaneously diabetic BB rat and NOD mouse (77). In the STZ-induced diabetic rat, enhanced TGF-β1 staining was noted in both the glomerulus and cortical tubules after 2 weeks of diabetes (80). A preliminary report found a positive correlation between glomerular monocyte infiltration, perhaps due to stimulation of monocyte chemotactic peptide (MCP-1), and glomerular TGF-β and fibronectin staining in STZ-induced diabetic rats at 7 and 14 days of diabetes (82). Another study reported similar results in STZ-induced diabetic rats at 14 and 30 days of diabetes (83).

Given that TGF-β1 is increased in temporal association with renal hypertrophy, we recently performed a preliminary interventional study using a neutralizing monoclonal antibody against TGF-β1, -β2, and -β3 in STZ-induced diabetic mice (69). Anti-TGF-β antibody or nonimmune murine IgG (300 μg/mouse) was administered intraperitoneally into either diabetic or nondiabetic mice on alternate days for a period of 8 days. Treatment with the neutralizing antibody reduced the increment in kidney weight by ~50% and prevented glomerular hypertrophy without having any effect on blood glucose concentration. mRNA levels for TGF-β1 and the type II TGF-β receptor were increased two- to threefold in the diabetic kidney cortex as early as 3 days after the onset of overt diabetes and coinciding with the onset of kidney enlargement. Moreover, α1(IV) collagen and fibronectin mRNA levels were increased severalfold in the diabetic kidney by the end of the study period. Treatment of diabetic mice with anti-TGF-β antibody significantly attenuated the increase in mRNAs encoding α1(IV) fibronectin. These results are the first to indicate that upregulation of TGF-β1 along with one of its signaling receptors in the diabetic kidney is responsible for the development of kidney and glomerular hypertrophy and for the increase in mRNAs encoding extracellular matrix components. Whether the progressive expansion of extracellular matrix in diabetic nephropathy has its origins in the first few days to weeks after the onset of diabetes remains unknown. Furthermore, whether the early upregulation of TGF-β expression in the glomeruli of diabetic kidneys is related to the subsequent development of diabetic glomerulosclerosis remains to be proven, but this relation is suggested by some studies (74,75,81). In STZ-induced diabetic rats, glomerular TGF-β1 mRNA is elevated after 12–24 weeks of diabetes (74), coincident with increased expression of α1(I), α1(III), and α1(IV) collagens, fibronectin, and laminins B1 and B2 mRNAs (81). Staining for glomerular TGF-β, fibronectin, and tenascin is also increased in long-term STZ-induced diabetes in rats (75).

**TGF-β and diabetic nephropathy**

**Human studies.** Only very limited studies have been performed on kidney tissue derived from patients with diabetic nephropathy. There is an association between enhanced glomerular TGF-β expression, as determined by immunohistochemical studies, and increased staining for fibronectin and tenascin in advanced diabetic nephropathy (75). Advanced disease is also associated with enhanced TGF-β expression in the tubulointerstitium (84).

**MECHANISMS OF TGF-β UPREGULATION IN DIABETIC KIDNEY DISEASE**

The factors mediating stimulation of glomerular TGF-β expression in the diabetic kidney probably involve hyperglycemia per se and/or insulin deficiency (as evidenced by cell culture studies [43] reviewed above), intraglomerular hemodynamic factors (85), the renin-angiotensin system (57,86,87), insulin-like growth factor (IGF-I) (88), or activation of PKC (8).

Angiotensin II (ANG II) stimulates renal tubular hypertrophy (57) and mesangial cell matrix overexpression (87) via autocrine stimulation of TGF-β in tissue culture, suggesting that part of the beneficial effect of ACEIs on diabetic renal hypertrophy (86) may well be due to inhibition of TGF-β activity. It should be noted that downregulation of ANG II receptors occurs in glomeruli (89,90) and tubules (91) from diabetic rats, consistent with chronic local overproduction of ANG II and a negative feedback effect on receptor expression. It is conceivable, therefore, that intrarenal ANG II production is enhanced and that ANG II stimulates TGF-β activity in the diabetic kidney despite long-term downregulation of ANG II receptors. Other evidence indicates that the prevailing high levels of TGF-β in the kidney may transform the mitogenic response to IGF-I into a hypertrophic response (92). This phenomenon is observed when proximal tubular cells are cultured in media containing TGF-β plus a high concentration of insulin (10 μg/ml) (92), which can activate IGF-I receptors (93).

The mechanism underlying high glucose–induced upregulation of the TGF-β1 system may involve PKC activation. Recent studies have convincingly demonstrated that high glucose increases diacylglycerol content in retinal cells, smooth muscle cells, and mesangial cells (8,94,95). PKC activation may mediate high glucose–induced matrix stimulation in mesangial cells (9,96). Activation of PKC usually leads to increased production of the Jun/Fos (API) transcription factor complex (97,98). The recent demonstration that glomerular mRNA abundance for both c-fos and c-jun is increased 24 h after induction of diabetes in the rat (99) lends credence to this hypothesis. The human promoter of TGF-β1 has multiple AP-1–like consensus sites that are responsive to phorbol-ester stimulation (100) and may mediate high glucose–induced gene transcription.

Increased stretching of mesangial cells and consequent release of TGF-β (101,102) may underlie the glomerulosclerosis that develops in hyperfiltering glomeruli with elevated glomerular capillary hydrostatic pressure (103). Additionally, hyperglycemia may enhance the glomerular expression of chemoattractants that enhance macrophage and platelet infiltration (82). Macrophages produce large quantities of TGF-β (83), and it is postulated that they may also stimulate parenchymal cells to produce TGF-β (104). The role of increased renin activity of the polyol pathway (12,105) on TGF-β expression in the diabetic kidney has not been evaluated. Other features of long-standing diabetes may also play an important role in stimulating the TGF-β system. The direct relationship between hyperglycemia and increased nonenzymatic glycation reactions has implicated excess protein glycation as a mechanistic link between hyperglycemia and the pathogenesis of diabetic nephropathy (10,11,106). The role of TGF-β in mediating the recently described nephropathic effects of Amadori-glycated proteins in diabetes (107) remains to be explored. A recent study has demonstrated that administration of advanced glycation end products (AGEs) to normal mice stimulates glomerular hypertrophy and glomerular TGF-β1, α1(IV) collagen, and laminin mRNA levels (108). Mesangial cells have specific receptors for AGEs (109) that may result in enhanced matrix
and cytokine production (109,110) including TGF-ß (79). However, the degree of TGF-ß1 stimulation (~50%) in glomeruli of AGE-treated normal mice is much less than previously reported in diabetic kidneys from mice and rats (three- to fivefold) (69,75,77,80,84).

Finally, the high glucose-induced stimulation of TGF-ß in renal cells may be related to the phenomenon of pseudohypoxia of hyperglycemia (111). This postulate can provide a unifying hypothesis regarding the pathogenesis of diabetic complications because it implicates the hypoxemia-induced increase in the redox state (e.g., high NADH-to-NAD+ ratio) in manifestations of diabetes in target tissues such as increased polyol pathway activity, increased de novo synthesis of diacylglycerol, and stimulation of PKC activity. The oxidative stress produced by nonenzymatic glycation may also be linked to the cellular dysfunction associated with the pseudohypoxic state (111). Note that studies in chronically ischemic myocardium in normal pigs have shown increased TGF-ß mRNA levels (112). However, evidence for a link between hyperglycemic pseudohypoxia and increased TGF-ß expression in the kidney will require additional studies.

**ATHERAPEUTIC APPROACHES TO ATTEMPT TGF-ß ACTIVITY**

Several strategies can be designed to interfere with the effect of TGF-ß in disease models. Most animal studies to date have used neutralizing antibodies to attenuate TGF-ß activity in a variety of disease states (49,68,113). Although this approach may have diagnostic or clinical use for short-term inhibition of TGF-ß activity, it would not be applicable to chronic diseases such as diabetic nephropathy.

The glomerular proteoglycan decorin binds TGF-ß and neutralizes its activity. Decorin has been used in different experimental models with varying results. In rats with glomerulonephritis induced by anti-Thy 1.1 antibody, decorin inhibits matrix deposition, presumably by interfering with TGF-ß activity (50). In a carotid artery injury model in rats characterized by upregulation of TGF-ß and matrix deposition, decorin does not appear to have a beneficial effect (113). In fact, decorin derived from bone tissue enhances TGF-ß bioactivity (114). It is likely that decorin has various effects depending on the target tissue, the dose used, and the degree of glycosylation of decorin derived from different sources (114). Since decorin is concentrated in the kidney, it may attain high concentrations that render TGF-ß inactive. When present at lower concentrations in other tissue sites, it may enhance presentation of active TGF-ß in the extracellular space to its cell-surface receptors.

The type III receptor, betaglycan, also can modify TGF-ß availability. When present in its membrane-bound form, it binds TGF-ß and presents it to the signal-transducing type I and type II receptors (115). However, when present in soluble form, it acts to inactivate TGF-ß and prevent interaction with its signaling receptors (22). Whether this property of soluble betaglycan can be used to therapeutic advantage remains to be explored.

Other therapeutic modalities may be useful. As discussed earlier, ANG II-induced stimulation of renal TGF-ß production in the diabetic kidney should be responsive to therapy with an ACEI. Aminoguanidine has been shown recently to decrease glomerular TGF-ß expression that was induced by administration of AGE into normal mice (108). Whether this effect of aminoguanidine is related to inhibition of AGE formation/action or to other unrelated mechanisms is unknown. Dietary protein restriction may have beneficial effects on diabetic nephropathy (116), but whether this response is related to inhibition of TGF-ß production is unclear. It is interesting, however, that two studies have demonstrated that a low-protein diet decreases glomerular TGF-ß expression in selected models of experimental glomerular and tubulointerstitial injury in rats (117,118).

**CONCLUDING REMARKS**

Much attention has been devoted recently to identifying an etiological role for one or more growth factors in many disease states including diabetes. Increased vascular-derived growth factor has been described in ocular fluid from patients with proliferative retinopathy due to diabetes (119). Deficiency of nerve growth factor may play a role in the inability of peripheral nerves to recover from injury caused by diabetic neuropathy (120). In the context of diabetic kidney disease, relevant growth factors that have been considered to be possible mediators include IGF-I, EGF, and PDGF (88,121–124), but the data to support a pathogenic role for a particular factor are inconclusive. The data we reviewed here argue strongly in support of a causal relationship between elevated production of TGF-ß in the kidney and diabetic renal disease. Novel means of interfering with the TGF-ß system in a selective tissue-specific manner are clearly required for long-term efficacy.

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