J Am Soc Nephrol 15: S55–S57, 2004

Mediators of Diabetic Renal Disease: The Case for TGF-β as the Major Mediator

FUAD N. ZIYADEH
Penn Center for Molecular Studies of Kidney Diseases, Renal-Electrolyte and Hypertension Division, Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Abstract. The critical role of hyperglycemia in the genesis of diabetic nephropathy has been established by cell culture studies, experimental animal models, and clinical trials. Certain cytokines and growth factors have been identified as likely mediators of the effects of high ambient glucose on the kidney, but prominent among these is TGF-β, a prototypical hypertrophic and fibrogenic cytokine. Overexpression of TGF-β has been demonstrated in the glomerular and tubulointerstitial compartments of experimental diabetic animals. The TGF-β receptor signaling system is also triggered, as evidenced by upregulation of the TGF-β type II receptor and activation of the downstream Smad signaling pathway. Treatment of diabetic mice with neutralizing anti–TGF-β antibodies prevents the development of renal hypertrophy, mesangial matrix expansion, and the decline in renal function. Antibody therapy also reverses the established lesions of diabetic glomerulopathy. These studies argue strongly in support of the hypothesis that overactivity of the TGF-β system in the kidney is a crucial mediator of diabetic renal hypertrophy and mesangial matrix expansion.

The structural renal changes in diabetes consist of glomerular and tubuloepithelial hypertrophy, followed by thickening of glomerular and tubular basement membranes and progressive accumulation of extracellular matrix proteins in the mesangium and the interstitium. Identifying mediators of increased synthesis or decreased degradation of matrix molecules in diabetic renal disease may help design novel, effective therapies to avert glomerulosclerosis and tubulointerstitial fibrosis and the development of proteinuria and progressive renal insufficiency.

TGF-β is one effector molecule that has been studied extensively as a major mediator of the hypertrophic and prosclerotic changes in diabetic kidney disease (1,2). TGF-β stimulates the synthesis of key extracellular matrix molecules including type I collagen, type IV collagen, fibronectin, and laminin (3). TGF-β also decreases matrix degradation by inhibiting proteases as well as activating protease inhibitors (*e.g.*, plasminogen activator inhibitor-1) (4). In addition, TGF-β promotes cell–matrix interactions by upregulating integrins, the cell surface receptors for matrix (5).

Almost all of the molecular mediators and intracellular signaling pathways that have been identified in diabetic kidney injury have also been found to stimulate the renal TGF-β activity as an intermediary step. These mediators encompass the following: high glucose concentration (6); early and advanced products of nonenzymatic glycation of proteins (7,8); oxidative stress and overproduction of superoxide by the mitochondrial electron-transport chain (9,10); cyclical stretch and relaxation of mesangial cells in culture (mimicking intraglomerular hypertension) (11); *de novo* synthesis of diacylglycerol and protein kinase C activation (12,13) and stimulation of mitogen-activated protein kinase (14); glucosamine overproduction (15); and high levels of vasoactive substances such as intrarenal angiotensin II (16), endothelin (17), and thromboxane (18).

High glucose concentration in cell culture studies stimulates hypertrophy of proximal tubular and mesangial cells (19,20) and stimulates the production of matrix molecules such as fibronectin and collagens in these cells as well as in epithelial, endothelial, and interstitial-fibroblastic cells (6,12,19,21–24). In almost all renal cell types, high ambient glucose upregulates the expression and bioactivity of TGF-β (6,22,25,26) and in some cases upregulates the TGF-β type II receptor (27). In fact, antagonism of TGF-β by specific neutralizing monoclonal antibodies (6) or by antisense oligonucleotides (28) virtually abolishes the high glucose-induced rise in matrix expression, indicating that TGF-β is a major mediator of the profibrotic effect of high glucose on the kidney.

Extensive evidence in experimental animal models of both type 1 and type 2 diabetes further implicates TGF-β as an important mediator of diabetic kidney disease. TGF-β1 mRNA and protein levels are increased in both the glomerular and tubular compartments of various models of experimental diabetes in rats and mice (29–33). It is interesting that upregulation of the renal TGF-β type II signaling receptor is concomitantly upregulated in diabetes (27,33–36). Recently, we reported significant activation in the diabetic kidney of the intracellular Smad pathway, which transduces the TGF-β signal (23,36). All of these studies provide evidence of activation

Correspondence to Dr. Fuad N. Ziyadeh, Renal-Electrolyte and Hypertension Division, University of Pennsylvania, 700 Clinical Research Building, 415 Curie Boulevard, Philadelphia, PA 19104-4218. Phone: 215-573-1837; Fax: 215-898-0189; E-mail: ziyadeh@mail.med.upenn.edu

1046-6673/1512-0055
Journal of the American Society of Nephrology

DOI: 10.1097/01.ASN.0000093460.24823.5B

of the TGF-β1 system and its intracellular signaling pathway in renal cell in diabetes.

Interventional studies in animals have shown that the development of diabetic renal disease is likely caused by the heightened activity of the renal TGF-β system. Short-term treatment of diabetic mice with neutralizing monoclonal antibodies against all three isoforms of TGF-β prevented glomerular hypertrophy and attenuated the increase in TGF-β1, α1(IV) collagen, and fibronectin mRNAs (34). Furthermore, long-term antibody therapy in the *db/db* mouse, a spontaneous model of type 2 diabetes, virtually prevented the mesangial matrix expansion and preserved the creatinine clearance (35). However, the anti–TGF-β antibody did not reduce albuminuria. Because the deleterious effects of proteinuria can be mediated by the TGF-β system itself (37), the apparent paradox of preserved renal function in the face of persistent albuminuria may be best explained by the effective inhibition of proteinuria-induced renal dysfunction with the neutralizing anti–TGF-β antibody regimen (2). Another likely possibility is that diabetic proteinuria, unlike other renal manifestations of diabetes, is mediated by increased vascular permeability factor/vascular endothelial growth factor (VEGF) rather than by TGF-β (24,35,38). Our preliminary data have shown upregulation of VEGF in the kidney of *db/db* mice and that treatment with anti–TGF-β antibody was ineffective in reducing VEGF levels (35).

Most recently, we addressed the question of whether anti–TGF-β therapy can *reverse* the histologic lesions of diabetic glomerulopathy once they are established (39). Diabetic *db/db* mice and their nondiabetic *db/m* littermates were allowed to grow until 16 wk of age, by which time the *db/db* mice had developed glomerular basement membrane (GBM) thickening and mesangial matrix expansion. The mice were then treated with an irrelevant control IgG or a panselective, neutralizing anti–TGF-β antibody for 8 more weeks. Compared with control *db/db* mice, the *db/db* mice that were treated with anti–TGF-β antibody showed attenuation of GBM thickening and regression of mesangial matrix accumulation (39). Thus, inhibiting renal TGF-β activity can partially reverse the GBM thickening and mesangial matrix expansion in this mouse model of type 2 diabetes.

Corroborative surveys of kidney specimens derived from patients with diabetes have also suggested an important role of the renal TGF-β system in the pathogenesis of diabetic renal disease (40,41). For instance, glomerular TGF-β1 mRNA is markedly increased in renal biopsy specimens from patients with proven diabetic kidney disease (42). In another study, we have shown that the gradient of TGF-β1 concentration across the renal vascular bed (venous minus arterial) was positive and the urinary level of bioassayable TGF-β was significantly increased in diabetic patients, indicating net production of TGF-β1 protein by the kidney in diabetes (43). Of clinical interest is the observation that angiotensin-converting enzyme inhibitor therapy may protect the kidney by lowering TGF-β1 production. In captopril-treated patients, especially in the subset of patients with an initial GFR of $<$75 ml/min, we demonstrated that a decrease in the circulating TGF-β1 level predicted a better preservation of the GFR (44).

In summary, the studies reviewed herein indicate that if novel therapies can be designed to intercept effectively the renal TGF-β axis, then it may be possible in the future to prevent, arrest, or even reverse the damaging effects of diabetes on the kidney.

Acknowledgments

Dr. Fuad N. Ziyadeh is supported by the Juvenile Diabetes Foundation and National Institutes of Health (NIH) grants DK-44513, DK-45191, and DK-54608.

References

1. Ziyadeh FN: Evidence for the involvement of transforming growth factor-β in the pathogenesis of diabetic kidney disease: Are Koch's postulates fulfilled? *Curr Pract Med* 1: 87–89, 1998
2. Reeves WB, Andreoli TE: Transforming growth factor beta contributes to progressive diabetic nephropathy. *Proc Natl Acad Sci U S A* 97: 7667–7669, 2000
3. Sharma K, Ziyadeh FN: Biochemical events and cytokine interactions linking glucose metabolism to the development of diabetic nephropathy. *Semin Nephrol* 17: 80–92, 1997
4. Ziyadeh FN, Sharma K: Role of transforming growth factor-beta in diabetic glomerulosclerosis and renal hypertrophy. *Kidney Int Suppl* 51: S34–S36, 1995
5. Heino J, Ignotz RA, Hemler ME, Crouse C, Massague J: Regulation of cell adhesion receptors by transforming growth factor-β. Concomitant regulation of integrins that share a common β1 subunit. *J Biol Chem* 264: 380–388, 1989
6. Ziyadeh FN, Sharma K, Ericksen M, Wolf G: Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by autocrine activation of transforming growth factor-β. *J Clin Invest* 93: 536–542, 1994
7. Yang C-W, Vlassara H, Peten EP, He C-J, Striker GE, Striker LJ: Advanced glycation end products up-regulate gene expression found in diabetic glomerular disease. *Proc Natl Acad Sci U S A* 91: 9436–9440, 1994
8. Ziyadeh FN, Han DC, Cohen JA, Guo J, Cohen MP: Glycated albumin stimulates fibronectin gene expression in glomerular mesangial cells: Involvement of the transforming growth factor-beta system. *Kidney Int* 53: 631–638, 1998
9. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414: 813–820, 2001
10. Iglesias-de la Cruz MC, Ruiz-Torres MP, Alcamí J, Díez-Marqués ML, Ortega-Velázquez R, Chen S, Rodríguez-Puyol M, Ziyadeh FN, Rodríguez-Puyol D: Hydrogen peroxide increases extracellular matrix mRNA through TGF-b in human mesangial cells. *Kidney Int* 59: 87–95, 2001
11. Riser BL, Cortes P, Heilig C, Grondin J, Ladson-Wofford S, Patterson D, Narins RG: Cyclic stretching force selectively upregulates transforming growth factor-beta isoforms in cultured rat mesangial cells. *Am J Pathol* 148: 1915–1923, 1996
12. Fumo P, Kuncio GS, Ziyadeh FN: PKC and high glucose stimulate collagen alpha1(IV) transcriptional activity in a reporter mesangial cell line. *Am J Physiol* 267: F632–F638, 1994
13. Koya D, Jirousek MR, Lin YW, Ishii H, Kuboki K, King GL: Characterization of protein kinase C beta isoform activation on the gene expression of transforming growth factor-β, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. *J Clin Invest* 100: 115–126, 1997

14. Isono M, Iglesias-de la Cruz MC, Chen S, Hong SW, Ziyadeh F: Extracellular signal-regulated kinase mediates stimulation of TGF-β1 and matrix by high glucose in mesangial cells. *J Am Soc Nephrol* 11: 2222–2230, 2000
15. Kolm-Litty V, Sauer U, Nerlich A, Lehmann R, Schleicher ED: High glucose-induced transforming growth factor β1 production is mediated by the hexosamine pathway in porcine glomerular mesangial cells. *J Clin Invest* 101: 160–169, 1998
16. Wolf G, Ziyadeh FN: The role of angiotensin II in diabetic nephropathy: Emphasis on nonhemodynamic mechanisms. *Am J Kidney Dis* 29: 153–163, 1997
17. Nakamura T, Ebihara I, Fukui M, Tomino Y, Koide H: Effect of a specific endothelin receptor A antagonist on mRNA levels for extracellular matrix components and growth factors in diabetic glomeruli. *Diabetes* 44: 895–899, 1995
18. Studer RK, Negrete H, Craven PA, DeRubertis FR: Protein kinase C signals thromboxane induced increases in fibronectin synthesis and TGF-beta bioactivity in mesangial cells. *Kidney Int* 48: 422–430, 1995
19. Ziyadeh FN, Snipes ER, Watanabe M, Alvarez RJ, Goldfarb S, Haverty TP: High glucose induces cell hypertrophy and stimulates collagen gene transcription in proximal tubule. *Am J Physiol* 259: F704–F714, 1990
20. Wolf G, Sharma K, Chen Y, Ericksen M, Ziyadeh FN: High glucose-induced proliferation in mesangial cells is reversed by autocrine TGF-beta. *Kidney Int* 42: 647–656, 1992
21. Wakisaka M, Spiro MJ, Spiro RG: Synthesis of type VI collagen by cultured glomerular cells and comparison of its regulation by glucose and other factors with that of type IV collagen. *Diabetes* 43: 95–103, 1994
22. Han DC, Isono M, Hoffman BB, Ziyadeh FN: High glucose stimulates proliferation and collagen type I synthesis in renal cortical fibroblasts: mediation by autocrine activation of TGF-beta. *J Am Soc Nephrol* 10: 1891–1899, 1999
23. Isono M, Chen S, Hong SW, Iglesias-de la Cruz MC, Ziyadeh FN: Smad pathway is activated in the diabetic mouse kidney and Smad3 mediates TGF-beta-induced fibronectin in mesangial cells. *Biochem Biophys Res Commun* 296: 1356–1365, 2002
24. Iglesias-de la Cruz MC, Ziyadeh FN, Isono M, Kouahou M, Han DC, Kalluri R, Mundel P, Chen S: Effects of high glucose and TGF-β1 on the expression of collagen IV and vascular endothelial growth factor in mouse podocytes. *Kidney Int* 62: 901–913, 2002
25. Hoffman BB, Sharma K, Zhu Y, Ziyadeh FN: Transcriptional activation of transforming growth factor-β1 in mesangial cell culture by high glucose concentration. *Kidney Int* 54: 1107–1116, 1998
26. Rocco MV, Chen Y, Goldfarb S, Ziyadeh FN: Elevated glucose stimulates TGF-β gene expression and bioactivity in proximal tubule. *Kidney Int* 41: 107–114, 1992
27. Isono M, Mogyorosi A, Han DC, Hoffman BB, Ziyadeh FN: Stimulation of TGF-beta type II receptor by high glucose in mouse mesangial cells and in diabetic kidney. *Am J Physiol Renal Physiol* 278: F830–F838, 2000
28. Han DC, Hoffman BB, Hong SW, Guo J, Ziyadeh FN: Therapy with antisense TGF-β1 oligodeoxynucleotides reduces kidney weight and matrix mRNAs in diabetic mice. *Am J Physiol* 278: F628–F634, 2000
29. Nakamura T, Fukui M, Ebihara I, Osada S, Nagaoka I, Tomino Y, Koide H: mRNA expression of growth factors in glomeruli from diabetic rats. *Diabetes* 42: 450–456, 1993
30. Sharma K, Ziyadeh FN: Renal hypertrophy is associated with upregulation of TGF-β1 gene expression in diabetic BB rat and NOD mouse. *Am J Physiol* 267: F1094–F1101, 1994
31. Yang C-W, Hattori M, Vlassara H, He C-J, Carome MA, Yamato E, Elliot S, Striker G, Striker LJ: Overexpression of transforming growth factor-β1 mRNA is associated with up-regulation of glomerular tenascin and laminin gene expression in nonobese diabetic mice. *J Am Soc Nephrol* 5: 1610–1617, 1995
32. Gilbert RE, Cox A, Wu LL, Allen TJ, Hulthen UL, Jerums G, Cooper ME: Expression of transforming growth factor-β1 and type IV collagen in the renal tubulointerstitium in experimental diabetes: Effects of ACE inhibition. *Diabetes* 47: 414–422, 1998
33. Hill C, Flyvbjerg A, Gronbaek H, Petrik J, Hill DJ, Thomas CR, Sheppard MC, Logan A: The renal expression of transforming growth factor-beta isoforms and their receptors in acute and chronic experimental diabetes in rats. *Endocrinology* 141: 1196–1208, 2000
34. Sharma K, Jin Y, Guo J, Ziyadeh FN: Neutralization of TGF-β by anti-TGF-β antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. *Diabetes* 45: 522–530, 1996
35. Ziyadeh FN, Hoffman BB, Han DC, Iglesias-De La Cruz MC, Hong SW, Isono M, Chen S, McGowan TA, Sharma K: Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice. *Proc Natl Acad Sci U S A* 97: 8015–8020, 2000
36. Hong SW, Isono M, Chen S, Iglesias-De La Cruz MC, Han DC, Ziyadeh FN: Increased glomerular and tubular expression of transforming growth factor-β1, its type II receptor, and activation of the Smad signaling pathway in the db/db mouse. *Am J Pathol* 158: 1653–1663, 2001
37. Remuzzi G, Bertani T: Pathophysiology of progressive nephropathies. *N Engl J Med* 339: 1448–1456, 1998
38. De Vriese AS, Tilton RG, Elger M, Stephan CC, Kriz W, Lameire NH: Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. *J Am Soc Nephrol* 12: 993–1000, 2001
39. Chen S, Iglesias-de la Cruz MC, Jim B, Hong SW, Isono M, FN Z: Reversibility of established diabetic glomerulopathy by anti-TGF-beta antibodies in db/db mice. *Biochem Biophys Res Commun* 300: 16–22, 2003
40. Yoshioka K, Takemura T, Murakami K, Okada M, Hino S, Miyamoto H, Maki S: Transforming growth factor-β protein and mRNA in glomeruli in normal and diseased human kidneys. *Lab Invest* 68: 154–163, 1993
41. Yamamoto T, Noble NA, Cohen AH, Nast CC, Hishida A, Gold LI, Border WA: Expression of transforming growth factor-β isoforms in human glomerular diseases. *Kidney Int* 49: 461–469, 1996
42. Iwano M, Kubo A, Nishino T, Sato H, Nishioka H, Akai Y, Kurioka H, Fujii Y, Kanauchi M, Shiiki H, Dohi K: Quantification of glomerular TGF-β1 mRNA in patients with diabetes mellitus. *Kidney Int* 49: 1120–1126, 1996
43. Sharma K, Ziyadeh FN, Alzahabi B, McGowan TA, Kapoor S, Kurnik BRC, Kurnik PB, Weisberg LS: Increased renal production of transforming growth factor-β1 in patients with type II diabetes. *Diabetes* 46: 854–859, 1997
44. Sharma K, Eltayeb BO, McGowan TA, Dunn SR, Alzahabi B, Rohde R, Ziyadeh FN, Lewis EJ: Captopril-induced reduction of serum levels of transforming growth factor-b1 correlates with long-term renoprotection in insulin-dependent diabetic patients. *Am J Kidney Dis* 34: 818–823, 1999