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TGF-β: A CRUCIAL COMPONENT OF THE PATHOGENESIS OF DIABETIC NEPHROPATHY

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INTRODUCTION

Recent studies involving tight blood glucose control and aggressive antihypertensive therapy especially with angiotensin converting enzyme (ACE) inhibitors have shown that the progression of diabetic nephropathy to an end-stage condition may be favorably modified if not entirely abrogated. Despite this, about 30% of all diabetic patients will develop nephropathy after a period of about 15 to 20 years. In the Diabetes Control and Complications Trial, 16 percent of normoalbuminuric patients with type 1 diabetes mellitus progressed to microalbuminuria over 10 years despite good blood glucose control (1). Once overt nephropathy occurs, tight glycemic control, difficult as it is to maintain, may not improve the course of the disease (1). Similarly, treatment with an ACE inhibitor markedly delays the onset of renal failure but does not totally prevent it.

In order to develop more effective treatments, we must develop a better understanding of the pathogenesis of diabetic nephropathy (2). We developed a renal cell culture system (3,4) to study the influence of high glucose on cell growth and extracellular matrix metabolism.

RESULTS AND DISCUSSION

The intra-renal effects of both hyperglycemia and angiotensin II could be the result of the action of transforming growth factor-β (TGF-β), a hypertrophic and prosclerotic cytokine (5). TGF-β stimulates the synthesis of extracellular matrix molecules. In addition, through its action to stimulate protease inhibitors (e.g., plasminogen activator inhibitors) and matrix-degrading proteases (e.g., collagenase and stromelysin) inhibits the breakdown of extracellular matrix. Hypergly-

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cemia (6–8), increased nonenzymatic glycation of proteins (9,10), stimulation of de novo synthesis of diacylglycerol and subsequent activation of protein kinase C (11), increased intracellular glucosamine production resulting from glucose metabolism (12), and enhanced renal production of vasoactive agents such as angiotensin II (5), endothelins (13), and thromboxane (14) all may be responsible for stimulation of the TGF-β system in diabetic nephropathy. Increased TGF-β1 production and bioactivity (15,16) occurs following intermittent cyclic stretching followed by relaxation of mesangial cells in culture (thereby mimicking the increased intraglomerular wall stress seen in vivo).

Koch's postulates (17) regarding the involvement of a cytokine in kidney diseases have all been fulfilled with regards to the TGF-β system and diabetic nephropathy:

1) Both high ambient glucose and recombinant TGF-β produce cell hypertrophy and increased extracellular matrix synthesis (18) in renal cells.
2) When a variety of renal cell-types are grown in high ambient glucose (including glomerular mesangial cells (7), proximal tubular epithelial cells (6), glomerular epithelial cells (19) and renal interstitial fibroblasts (20), increased amounts of TGF-β are produced with regulation primarily occurring at the level of gene transcription (21), at least in mesangial cells.
3) Both experimental diabetic animals (22,23) and humans with diabetes mellitus (24–26) manifest increased renal TGF-β expression (26).
4) Renal dysfunction characterized by proteinuria, glomerulosclerosis, and tubulointerstitial fibrosis is seen when transgenic mice overexpress active TGF-β1 in the liver (27) or in the liver and the proximal tubule (28) and when the glomerulus in the rat kidney is genetically manipulated to transiently overexpress TGF-β1 (29).
5) Inhibition of TGF-β bioactivity leads to reversal of the glucose-induced stimulation of collagen and fibronectin production in cultured renal cells (8,20,30).
6) The early manifestations of diabetic renal disease in mice can be prevented by systemic treatment with anti-TGF-β antibody (31) or antisense TGF-β1 oligodeoxynucleotides (32). Finally, the long-term study employing chronic anti-TGF-β antibody therapy in vivo has proved efficacious in preventing renal insufficiency in diabetic db/db mice (33).

In this long-term study (33), we tested the efficacy of a monoclonal, panselective neutralizing anti-TGF-β antibody or an irrelevant murine isotype-matched control IgG in db/db diabetic mice, a model of type 2

diabetes mellitus (33). After 8 weeks, control db/db mice developed proteinuria, doubling of plasma creatinine concentration, diffuse mesangial matrix expansion, and increased renal collagen IV and fibronectin mRNA levels. Treatment with anti-TGF-β antibody significantly decreased total plasma TGF-β1, attenuated the increase in plasma creatinine concentration, and markedly blunted the increase in collagen IV and fibronectin mRNA levels. This renoprotective effect of anti-TGF-β antibody was independent of any change in blood glucose. There were no noted ill effects of long-term treatment with the anti-TGF-β antibody. Taken together, these results provide the first evidence that the early as well as the late characteristic features of diabetic renal disease are largely mediated by an up-regulated renal TGF-β system.

As noted above and expanded upon below, there is a putative role for TGF-β in human diabetic nephropathy. Patients with overt nephropathy have increased glomerular and tubulointerstitial TGF-β immunostaining of all three TGF-β mammalian isoforms (24). Kidney biopsy specimens from patients with diabetic nephropathy display significantly increased glomerular TGF-β1 mRNA level, that correlates positively with the sclerosis index and level of glycosylated hemoglobin (25).

Aortic, renal vein, and urinary levels of TGF-β were measured in 14 type 2 diabetic and 11 non-diabetic patients undergoing elective cardiac catheterization for coronary artery disease. Renal blood flow was measured to calculate net mass balance across the kidney vascular bed. Diabetic patients demonstrated a higher venous-minus-arterial concentration of immunoreactive TGF-β1 than non-diabetic patients, and urinary levels of bioassayable TGF-β were also significantly higher in the diabetic patients (26).

Recent data also suggest that seemingly disparate factors that are thought to play a role in initiating diabetic nephropathy all converge to activate the TGF-β system in the kidney. These include intrarenal angiotensin II activation, oxidative stress, serum or matrix proteins that are modified by early and advanced glycation adducts, and activation of the polyol and glucosamine pathways (34–40).

SUMMARY AND CONCLUSIONS

In summary, metabolic, hemodynamic, and genetic factors are all important in the development and progression of diabetic nephropathy (33). Recent studies using cell culture techniques and experimental animal models have provided important insight into the role of hyper-

glycemia in this disease. The nature of the factors directly arising as a consequence of hyperglycemia and the steps involved in diabetic complications are not completely understood. The characteristic lesions of diabetic nephropathy may be intimately related to the effects of high ambient glucose on intracellular signaling events, various growth factors/cytokines, and nonenzymatic glycation of proteins (36). The growth factor TGF-β has emerged as a key participant in the cascade of events which leads to kidney sclerosis. Increased TGF-β expression in the kidney in diabetes mellitus mediates the renal actions of high ambient glucose to promote cellular hypertrophy and stimulate extracellular matrix biosynthesis. Neutralizing the actions of TGF-β with highly specific monoclonal antibodies or with application of antisense technology can effectively prevent the induction of the kidney lesions of diabetes in mice independent of any changes in blood glucose levels. Such maneuvers are crucial for establishing proof-of-concept and Koch's postulates (17), but they are still far removed from clinical applicability. Nevertheless, it is hoped that further studies to elucidate the efficacy of novel interventions to intercept the activity of the renal TGF-β system will prove useful for effectively halting the progression of diabetic nephropathy.

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DISCUSSION

Mitch, Atlanta: More severe proteinuria can reflect something that is carried by the protein and absorbed by tubular epithelial cells to damage the cells. Alternately, the degree of proteinuria could reflect more severe injury in the kidney. Regardless, the disassociation you find between proteinuria and its effects on the glomerulus is quite interesting and needs further evaluation.

Glassock, Laguna Niguel: Stan, your presentations are a lot like your golf game—accurate and just the right amount of spin. I just wanted to ask you to speculate a little bit, because we all know diabetic nephropathy does not occur in all patients with diabetes. In part this is explained by better glucose control, but even after you factor for that, there appears to be a population of patients who seem to be resistant to the development of nephropathy. This has led to the suggestion that there may be a diabetic nephropathy gene, and because of certain ethnic distributions, perhaps that gene has a different prevalence in different ethnic populations; is there any evidence whatsoever that this difference in susceptibility is linked directly or indirectly to the ability to express TGF?

Goldfarb: There is one paper that I think is relevant to this question. Pociot et al (Pociot F, Hansen PM, Karlsen AE, Langdahl BL, Johannesen J, Nerup J: TGF-betal gene mutations in insulin-dependent diabetes mellitus and diabetic nephropathy. J Am Soc Nephrol 1998 Dec;9(12): 2302–7) suggests that a mutation in the TGF beta gene, (Thr263I1e) has a weak but significant correlation with diabetic nephropathy. This mutation was associated with differences in TGF-beta mRNA levels. I have no doubt that many more studies of the various proteins important in the phenotypic expression of TGF-beta action will be assessed in the future, including the role of the Smad proteins, transcription factors important in the regulation of TGF-beta gene expression.

Luke, Cincinnati: To continue with the parade of Nephrology questions, we are emphasizing to primary care physicians that they should follow particularly Type I, but probably also Type II diabetics and at the onset of microalbuminuria use of ace inhibitors, even in the absence of hypertension; what do you think microalbuminuria represents in a structural sense?

Goldfarb: Microalbuminuria is certainly an important marker of renal involvement and the greater the degree of microalbuminuria, the more likely that patients will go on to develop overt diabetic nephropathy. However, its structural correlates remain elusive. Recently, more attention has been paid to the podocytes, the epithelial-derived cells in the glomerulus, as potential keys to understanding proteinuria in general. Study of this cell type and its response to hyperglycemia may help answer your question.

Smith, Lawrence: With a common association of nephropathy and retinopathy in the diabetic, have any studies been done to look if this system is also involved in the retinopathy?

Goldfarb: While there is evidence that TGF-beta may also be involved in the pathogenesis of diabetic retinopathy, that condition is much more a result of neovascularization of the retina. It is likely that factors such as Vascular Endothelial Growth Factor (VEGF) and Insulin-Like Growth Factor-1 (IGF-1) play a more fundamental role in the creation of new retinal blood vessels and diabetic retinopathy.