Urinary tumor necrosis factor contributes to sodium retention and renal hypertrophy during diabetes

KEITH DIPIETRILLO, BONITA COUTERMARSH, AND FRANK A. GESEK
Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03755
Submitted 22 January 2002; accepted in final form 7 August 2002

DiPetrillo, Keith, Bonita Coutermarsh, and Frank A. Gesek. Urinary tumor necrosis factor contributes to sodium retention and renal hypertrophy during diabetes. Am J Physiol Renal Physiol 284: F113–F121, 2003. First published August 13, 2002; 10.1152/ajprenal.00026.2002.—Nephropathy is a major contributor to overall morbidity and mortality in diabetic patients. Early renal changes during diabetes include Na retention and renal hypertrophy. Tumor necrosis factor (TNF) is elevated during diabetes and is implicated in the pathogenesis of diabetic nephropathy. We tested the hypothesis that TNF contributes to Na retention and renal hypertrophy during diabetes. Rats with streptozotocin-induced diabetes exhibit increased urinary TNF excretion, Na retention, and renal hypertrophy through the first 20 days of diabetes. Administration of a soluble TNF antagonist (TNFR:Fc) to diabetic rats reduces urinary TNF excretion and prevents Na retention and renal hypertrophy. TNF stimulates Na uptake in distal tubule cells isolated from diabetic rats, providing a possible mechanism for TNF-induced Na retention. We conclude that urinary TNF contributes to early diabetic nephropathy and may serve as a valuable diagnostic marker. Furthermore, inhibition of TNF during diabetes may attenuate early pathological changes in diabetic nephropathy.

distal tubule; albuminuria; sodium retention; hypertrophy; TNFR:Fc

DIABETES IS THE LEADING CAUSE of end-stage renal disease in the United States and Europe (24). Diabetic nephropathy contributes substantially to overall morbidity and mortality in diabetic patients, either directly or indirectly as a risk factor for cardiovascular disease. Microalbuminuria (defined as urinary albumin-to-creatinine ratio of 300 mg/g) is the earliest clinical sign of diabetic nephropathy (24) and is associated with an accelerated decline in glomerular filtration rate in diabetic patients compared with normoalbuminuric patients (24, 33). Microalbuminuria is an independent risk factor for the development of ischemic heart disease (4) and cardiovascular events (myocardial infarction, stroke, hospitalization for congestive heart failure, or death) (11). Nephropathy is associated with left ventricular hypertrophy and impaired diastolic relaxation in type 1 diabetic patients (39). Nephropathy also underlies the development of hypertension in type 1 diabetic patients (24). Improved understanding of the early pathological changes in diabetic nephropathy may identify novel clinical markers for detecting early renal disease, as well as elucidate new therapeutic strategies to diminish these changes and reduce the progression to end-stage renal disease.

The principal renal alterations that occur during the initial stage of diabetic nephropathy are hypertrophy and hyperfunction (see Ref. 23 for review). Renal enlargement is common in diabetic patients (3, 21) and may predict progression to overt diabetic nephropathy (3). Na retention is a manifestation of hyperfunction and is observed in diabetic patients before the onset of albuminuria (29, 41, 45). Na retention likely contributes to the onset of hypertension (41, 44, 46), which develops from underlying renal dysfunction in type 1 diabetic patients (24). Experimental evidence suggests that Na retention may also contribute to organ hypertrophy. Elevated dietary Na can lead to renal hypertrophy and induce transforming growth factor-β (TGF-β) expression in nondiabetic rats (48). TGF-β has been identified as a hypertrophic factor during diabetic nephropathy (see Ref. 36 for review). Moreover, Na restriction reduces renal hypertrophy in diabetic rats (1). Because hypertension (24) and renal hypertrophy (3, 21) are frequently observed in diabetic patients, Na retention may represent a significant manifestation of altered renal function that occurs during diabetes.

Renal enlargement during early diabetic nephropathy results primarily from tubular hypertrophy (34). Proximal and distal tubules are enlarged as a result of hypertrophy and hyperplasia (34). Early degenerative changes in diabetes occur in the distal tubule (DT) and precede changes in proximal tubules (PT) and glomeruli (47). Tubular function is also altered early in the course of diabetes. Urinary excretion of α1-microglobulin and Tamm-Horsfall protein (THP), markers of PT and DT dysfunction, respectively, are increased in diabetic patients before the development of albuminuria (35). Altered tubular function during diabetes likely contributes to Na retention.
The pathological events underlying the initial changes of diabetic nephropathy are not well defined. Inhibitory monoclonal antibodies against TGF-β attenuate kidney enlargement in diabetic mice, illustrating that TGF-β participates in renal hypertrophy during diabetes (50). Thomson et al. (43) proposed that hypertrophy of the PT early in diabetes increases reabsorption and, subsequently, elevates glomerular filtration rate. They demonstrated that enhanced ornithine decarboxylase activity contributes to renal hypertrophy and hyperfunction. However, inhibition of either pathway does not fully reduce renal hypertrophy, suggesting the existence of additional pathogenic mechanisms.

Tumor necrosis factor (TNF) is elevated during diabetes (2, 9, 22, 25), and TNF is implicated in the development of diabetic nephropathy (7, 18, 27, 42). In the present study, we tested the hypothesis that TNF underlies Na retention and renal hypertrophy during diabetes. To test this hypothesis, we administered a

METHODS

Animals. Twelve-week-old male Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA), and diabetes was induced by tail vein injection of streptozotocin (STZ, 50 mg/kg; Sigma, St. Louis, MO) dissolved in 0.9% sodium chloride. Blood glucose levels were measured before and 3–5 days after STZ injection to ensure the onset of hyperglycemia. Hyperglycemia was also confirmed by measuring urine glucose using Ketodastix (Bayer, Elkhart, IN) in rats housed in metabolic cages. Control and diabetic rats were treated with the anti-TNF agent TNFR:Fc, a soluble TNF receptor fusion protein. Subcutaneous TNFR:Fc (2 mg/kg; Immunex, Seattle, WA) injections started 1 day before STZ injection and continued twice weekly for 20 days.

Metabolic cage studies. Animals were housed in metabolic cages to determine whole animal Na balance. Food intake was determined by carefully weighing the amount of food provided to each rat housed in a metabolic cage. After 24 h, the remaining food was collected. Small particles of chow that fell through the floor of the metabolic cage were collected with feces by design of the metabolic cage. These small pieces were separated from feces, weighed with the remaining food, and subtracted from the starting weight to determine total food intake per 24 h. Na intake was subsequently calculated as a percentage of food intake (49) based on the Na content of the chow provided by the manufacturer (Harlan-Teklad, Madison, WI). All animals received the same lot of rat chow, ensuring consistent Na content in the food.

Water intake and urine output were also measured in animals housed in metabolic cages. Urine glucose and ketone concentrations were determined using Ketodastix. Urine Na, creatinine, and albumin concentrations were quantified at the clinical laboratory at Dartmouth-Hitchcock Medical Center (Lebanon, NH). Na balance was calculated as the difference between Na intake (determined as a percentage of food intake) and Na output (urine Na concentration × urine volume). This method has been used successfully to determine Na balance in rats (49). For albumin measurements, a standard curve (r² = 0.99) was generated using rat albumin (Sigma) with a lower limit of 5 μg/mL. At the conclusion of metabolic cage experiments (day 20), kidneys were excised and weighed to determine wet kidney weight, an established index of renal hypertrophy during diabetes (43, 50).

DT cell isolation. An in situ enzyme digestion procedure previously described was used to isolate PT and DT cells from diabetic and control rat kidneys (16). Briefly, kidneys were perfused with a mixture of collagenase (1 mg/ml) and hyaluronidase (3 mg/ml) and subsequently excised. The kidneys were sliced longitudinally, and the renal medulla was removed. The remaining renal cortex contained primarily PT and DT segments. The cortex was sliced and incubated with collagenase (1 mg/ml) for 10 min at 37°C. The mixture was filtered through wire mesh to remove tissue remnants, and the cells were incubated with a magnetic particle coupled to a primary antibody recognizing THP (Polyscience, Warrington, PA) (32). The flasks were affixed to a magnet to selectively remove THP-containing DT cells, comprising cells from the thick ascending limb and distal convoluted tubule (30, 40). The remaining cells represent primarily PT cells. DT cells isolated by this procedure exhibited characteristic DT Na and Ca transport pathways, including calcitonin- and parathyroid hormone-stimulated Ca transport (10, 13), amiloride- and chlorothiazide-inhibitable Na uptake (14), and phenylephrine-stimulated Na transport (12). The specificity of PT cells isolated by this method was confirmed by the presence of ethylisopropyl amiloride-inhibitable and chloridin-sensitive Na uptake (15).

Na uptake assay. To quantify Na entry, uptake of 22Na into isolated DT cells was measured using a rapid filtration technique described in previous reports (15). DT cells were incubated with recombinant rat TNF (Research Diagnostics, Flanders, NJ) at 37°C before addition of 22Na. Entry of 22Na into cells was terminated after 1 min by rapid addition of ice-cold isosmotic buffer, and cells were filtered onto Whatman GF/C filters using a Millipore 12-port manifold. Nonspecific binding of 22Na to filters and cells was determined and subtracted to calculate uptake. Uptake measurements were normalized to total cellular protein quantified by the Pierce bicinchoninic acid protein assay (Pierce, Rockford, IL), using BSA as a standard.

Detection of TNF and THP. Urine TNF levels were measured by ELISA using the OptEIA rat TNF set (Pharmingen, San Diego, CA) according to the accompanying protocol, using recombinant rat TNF as a standard. Urinary THP was also measured by ELISA. Briefly, Nunc Maxisorp 96-well ELISA plates were coated with goat anti-human THP antibody (ICN, Irvine, CA; 1:1,000) diluted in coating buffer (0.1 M carbonate, pH 9.5). Urine samples were incubated at room temperature for 3 h, each well was washed, and horseradish peroxidase-conjugated sheep anti-human THP antibody (Biogenesis, Kingston, NH) was added for 1 h. A 3,3',5,5'-tetramethylbenzidine-based substrate set (Pharmingen) was used for color development. A standard curve using rat THP (Biogenesis) was generated for each experiment.

Immunofluorescence and confocal microscopy. DT cells were grown on Transwell filters to maintain membrane polarization (Costar, Cambridge, MA) for confocal microscopy or on glass coverslips for fluorescence microscopy. The cells were fixed with methanol-free paraformaldehyde in phosphate-buffered saline solution. DT cells were incubated overnight at 4°C with primary antibodies to TNF type 1 (TNFR1) or type 2 receptor (TNFR2; Santa Cruz Biotechnology, Santa Cruz, CA). DT cells were incubated with secondary antibody

TNF CONTRIBUTES TO EARLY DIABETIC NEPHROPATHY
coupled to Alexa 568 (Molecular Probes, Eugene, OR) and treated with ProLong Antifade reagent (Molecular Probes). Confocal images were captured using a Zeiss microscope attached to a scanning confocal system (model MRC-1024, Bio-Rad) followed by three-dimensional reconstruction using Bio-Rad imaging software. Immunofluorescence images were visualized using a fluorescence microscope (Olympus America, Melville, NY).

Statistical analysis. Metabolic cage data for each treatment group are presented as means ± SE for control (n = 3), diabetic (n = 8), diabetic + TNFR:Fc (n = 9), and control + TNFR:Fc (n = 3). Statistical significance was determined using ANOVA followed by a Student-Newman-Keuls multiple comparisons test. P < 0.05 was considered significant.

RESULTS

Urinary TNF excretion is increased during diabetes. Renal TNF expression is increased in animal models of diabetes (26, 42). To determine whether renal TNF protein is increased and released during diabetes, urinary TNF excretion was measured. A significant increase in urinary TNF excretion was observed within the first 3 days after induction of diabetes (Fig. 1). Elevated urinary TNF excretion continued through the first 20 days of diabetes. Urinary TNF excretion was also measured in diabetic + TNFR:Fc rats. TNFR:Fc is a soluble TNF antagonist consisting of two TNFR2 extracellular domains fused to the Fc portion of human IgG1 (5). TNFR:Fc binds to soluble TNF and prevents TNF from interacting with its cognate cell surface receptors. In diabetic + TNFR:Fc rats, TNFR:Fc administration significantly reduced urinary TNF excretion, nearly to control levels, throughout the 20-day study period (Fig. 1). TNFR:Fc does not alter the metabolic profile of diabetic rats. To accurately determine the role of TNF in the development of diabetic nephropathy, we first determined whether TNF inhibition alleviated the metabolic abnormalities of diabetes, because improved metabolic control could prevent the development of diabetic nephropathy (8, 24, 37). Urine glucose was significantly enhanced in diabetic rats from day 3 through day 20 (Fig. 2A). TNFR:Fc therapy did not affect urinary glucose at any day of the study period. The urinary ketone concentration in the diabetic group was not significantly increased above control until days 17 and 20 (Fig. 2B). TNFR:Fc administration did not alter ketonuria in diabetic rats throughout the first 17 days of diabetes but did significantly reduce ketonuria at day 20 (Fig. 2B). Water intake, food intake, and urine output were increased equally in the diabetic and diabetic + TNFR:Fc groups (Fig. 2, C–E). Although food intake increased, diabetic rats lost weight during the initial week of diabetes and subsequently maintained stable body weight throughout the rest of the study period (Fig. 2F). TNFR:Fc treatment did not alter body weight changes during diabetes. Urine glucose and ketones were virtually undetectable in control rats. Water intake, urine output, and food intake remained stable in control rats as they progressively gained body weight throughout the 20-day study period. TNFR:Fc administration did not affect these metabolic indexes in control + TNFR:Fc rats.

TNFR:Fc reduces Na retention in diabetic rats. To investigate the role of TNF in the maintenance of Na homeostasis during diabetes, we measured dietary Na intake and urinary Na output in diabetic and diabetic + TNFR:Fc rats. Dietary Na intake remained stable in control and control + TNFR:Fc rats throughout the 20-day study period (Fig. 3A). In contrast, dietary Na intake was increased above control equally in diabetic and diabetic + TNFR:Fc rats starting at day 7 and continuing through day 20 (Fig. 3A).

Urinary Na output was constant throughout the study period in control rats (Fig. 3B). TNFR:Fc therapy did not alter urinary Na output in control + TNFR:Fc rats. Na output was elevated in diabetic rats compared with controls, probably because of markedly increased dietary Na intake in diabetic rats (Fig. 3B). TNFR:Fc administration significantly enhanced urinary Na output in diabetic + TNFR:Fc rats throughout the first 17 days of the study period (Fig. 3B). The ability of TNFR:Fc to augment urinary Na excretion during diabetes was independent of therapeutic effects on the metabolic state of the animals, because TNFR:Fc did not alter metabolic indexes of diabetes through day 17 (Fig. 2).

After quantifying dietary Na intake and urinary Na output, we calculated the difference between Na intake and output to determine Na balance. Na balance was stable in control and control + TNFR:Fc rats during the study period (Fig. 3C). Diabetic rats exhibited significant Na retention compared with control as early as day 7 of diabetes and continued to retain Na through day 20 of the study. TNFR:Fc treatment significantly reduced Na retention in diabetic + TNFR:Fc rats (Fig. 3C).
TNFR:Fc reduces renal hypertrophy during diabetes. Experimental studies demonstrate that excess Na contributes to renal hypertrophy (1, 48). Increased kidney size is common in diabetic patients (3, 21). On the basis of our findings that TNF inhibition reduced Na retention during diabetes, we tested the hypothesis that TNF inhibition would reduce renal hypertrophy during diabetes. As an index of renal hypertrophy, we measured average wet kidney weight from TNFR:Fc-treated and untreated control and diabetic rats. Wet kidney weight is an accurate measure of renal hypertrophy during diabetes (43, 50). Kidney weight was significantly increased in diabetic rats (day 20) compared with controls (Fig. 4). TNFR:Fc did not affect wet kidney weight in control + TNFR:Fc rats. In contrast, TNFR:Fc therapy significantly reduced renal hypertrophy in diabetic + TNFR:Fc rats (Fig. 4).

Na retention and renal hypertrophy precede albuminuria. The development of microalbuminuria, defined as urinary albumin-to-creatinine ratio of 30–300 mg/g (24), is used as a marker of glomerular dysfunction during diabetes (24). Albuminuria is the earliest clinical marker of diabetic nephropathy and correlates with progression to end-stage renal disease (24, 33) and cardiovascular morbidity and mortality (4, 11, 39). We measured urinary albumin-to-creatinine ratios on days 10 and 20 after the onset of diabetes to determine whether Na retention and renal hypertrophy precede albuminuria during diabetes. Control rats did not exhibit measurable urinary albumin excretion on day 10 or 20. None of the diabetic rats displayed microalbuminuria on day 10, at which point Na retention was established. On day 20, when diabetic rats exhibited significant renal hypertrophy, only two of eight diabetic rats exhibited microalbuminuria (albumin-to-creatinine ratios of 191.3 and 146.0 mg/g), whereas the remaining diabetic rats were normoalbuminuric.

TNF stimulates Na uptake in DT cells isolated from diabetic rats. Tubular structure and function are altered in the early stages of diabetes (31). To determine
whether TNF induces Na retention by stimulating tubular Na uptake, we measured Na uptake in PT and DT cells freshly isolated from control and diabetic rats. Basal Na uptake in PT cells was unchanged after 10 days of diabetes (Fig. 5A). Acute exposure to 10 or 100 ng/ml TNF did not stimulate Na uptake in PT cells isolated from control or diabetic rats (Fig. 5A).

DT function is important for the regulation of Na homeostasis and is altered during diabetes (31, 35). We confirmed DT tubule dysfunction in our animal model of diabetes by measuring urinary THP excretion by ELISA. Diabetic rats exhibited a significant increase in THP excretion from day 3 through day 20 of the study period (data not shown). Because DT function was altered early in diabetes, we hypothesized that TNF induced Na retention by stimulating Na transport in DT cells. To test this hypothesis, we isolated DT cells from control rats or after 10 or 20 days of diabetes and quantified TNF-stimulated Na uptake. Basal rates of Na transport were unaltered in DT cells after 10 or 20 days of diabetes (Fig. 5B; control basal rate = 256.6 ± 5.7 nmol·min⁻¹·mg protein⁻¹). TNF had no effect on Na transport in DT cells from control rats. In contrast, TNF stimulated Na uptake in DT cells isolated from diabetic rats after 10 and 20 days. This differential activation of Na transport by TNF occurred over a wide concentration range. TNF at 10 ng/ml stimulated Na uptake in DT cells from diabetic rats, whereas DT cells from control rats responded minimally to TNF concentrations up to 1 μg/ml (Fig. 5C).

Expression and cellular localization of TNF receptors. TNF modulates cellular function through distinct membrane receptors, TNFR1 and TNFR2 (see Ref. 38 for review). To examine the expression and cellular localization of TNF receptors in DT cells, immunofluorescence and confocal studies were conducted with antibodies specific for TNFR1 and TNFR2 (6). In immunofluorescence studies using nonpolarized DT cells grown on glass coverslips, TNFR1 and TNFR2 were observed in DT cells isolated from control rats (Fig. 6). TNFR1 and TNFR2 were also expressed in DT cells isolated from diabetic rats (data not shown). TNF re-

Fig. 3. TNFR:Fc augments urinary Na output and reduces Na retention during diabetes. A and B: effect of TNFR:Fc treatment on dietary Na intake and urinary Na output in control and diabetic rats. C: effect of TNFR:Fc therapy on Na balance in control and diabetic rats. Na balance was calculated as the difference between dietary Na intake and urinary Na output for individual rats on each day. Values are means ± SE of 3 Cont and Cont + TNFR:Fc, 8 Diab, and 9 Diab + TNFR:Fc rats. *P < 0.05 vs. Diab.

Fig. 4. TNFR:Fc prevents renal hypertrophy during diabetes. Effect of TNFR:Fc treatment on wet kidney weight is shown in control and diabetic (day 20) rats. Values are means ± SE of 3 Cont and Cont + TNFR:Fc, 8 Diab, and 9 Diab + TNFR:Fc rats. *P < 0.05 vs. Cont.
ceptor localization was examined by confocal microscopy using DT cells grown on filters to maintain polarity of apical and basal membranes. DT cells are polarized in vivo, such that the apical membrane contacts the urine and the basal membrane faces the blood. In confocal images, TNFR1 was clearly localized to the apical portion of DT cells, whereas TNFR2 was expressed diffusely throughout the cell (Fig. 6). Membrane localization of either TNF receptor subtype was not altered during diabetes (data not shown).

DISCUSSION

The focus of this study was to elucidate the role of TNF in the development of early diabetic nephropathy. Our results implicate TNF as an important factor underlying diabetic nephropathy. Diabetic rats exhibited enhanced urinary TNF excretion, Na retention, and renal hypertrophy. Administration of the TNF antagonist TNFR:Fc significantly decreased urinary TNF and prevented Na retention and renal hypertrophy. These observations imply that enhanced TNF production and release are necessary to induce Na retention and renal hypertrophy in diabetes.

The mechanism of TNF-induced Na retention during diabetes is unclear, although several findings suggest that augmented tubular Na transport underlies Na retention in response to elevated TNF. First, TNF directly stimulated Na uptake in DT cells isolated from diabetic rats. Second, TNFR:Fc treatment augmented urinary Na output during diabetes compared with untreated diabetic rats, despite equivalent Na and water intake and urine output, implying that TNFR:Fc inhibited tubular Na transport. Finally, TNF was undetectable in serum samples from any of the groups, whereas urinary TNF was markedly increased in diabetic rats, suggesting that TNF actions were mediated from the lumen of the nephron. Taken together, these findings implicate enhanced tubular Na transport as the mechanism underlying TNF-induced Na retention.

The role of DT cells underlying Na retention during diabetes is intriguing. Our data show that DT cells are sensitized to the acute effects of TNF on Na transport during diabetes. TNF does not activate Na uptake in DT cells isolated from control rats, but TNF significantly increases Na transport in DT cells from diabetic rats. The factor(s) leading to DT sensitization during diabetes is unclear but may include increased urinary TNF or glucose excretion. Second, DT cells normally reabsorb only a small percentage of the filtered Na load but are critical for the fine regulation of Na homeostasis. Our findings show that perturbations in DT Na transport may induce profound changes in whole animal Na retention. However, TNFR:Fc treatment does not fully reverse Na retention in diabetic rats, which suggests that additional mechanisms may in part contribute to Na retention. The ornithine decarboxylase-mediated increase in PT Na reabsorption during diabetes may contribute to net Na retention (43). Alternatively, elevated urinary glucose concentrations may enhance proximal tubule Na reabsorption via constitutively expressed Na-glucose transporters.

TNF contributes to Na retention and renal hypertrophy in the early stages of diabetes; however, the mechanisms for TNF actions have not been determined. Our experiments do not fully differentiate between renal hypertrophy induced by direct TNF actions and effects

![Fig. 5. TNF stimulates Na uptake selectively in distal tubule (DT) cells during diabetes. A: active Na transport in proximal tubule cells isolated from control and diabetic (day 10) rats (Diab 10d). Cells were exposed to TNF for 15 min. Values are means ± SE of triplicate determinations of each experimental condition for 3 rats. B: basal and TNF-stimulated (10 ng/ml; 15 min) Na uptake in DT cells isolated from control and diabetic rats. C: TNF-stimulated Na uptake is selective in DT cells from diabetic rats over a wide dose range. Values are means ± SE for triplicate determinations of 5 diabetic rats on day 20 (Diab 20d) or 6 Cont and Diab 10d rats. *P < 0.05 vs. Cont. #P < 0.05 vs. basal.](image-url)
secondary to Na retention. High-Na feeding can induce renal TGF-β expression and hypertrophy in rats (48). In theory, the hypertrophic effects of TNF during diabetes could be mediated by Na retention, with subsequent TGF-β effects. Experiments to inhibit Na retention without affecting urinary TNF excretion in diabetes are needed to distinguish between these possibilities. At the cellular level, TNF-stimulated Na uptake could be mediated by bumetanide-sensitive Na-K-2Cl cotransporters in thick ascending limb cells or by amiloride-sensitive epithelial Na channels or thiazide-inhibitable Na-Cl cotransporters in distal cortical tubule cells. In other studies, we show that TNF-stimulated Na uptake was inhibited by amiloride, implicating the epithelial Na channel as the Na transport mechanism underlying TNF-stimulated Na uptake in DT cells (unpublished observations).

Elevated urinary TNF correlated with Na retention and renal hypertrophy, and reduction of urinary TNF with TNFR:Fc therapy ameliorated Na retention and hypertrophy. Serum TNF was undetectable in all the treatment groups, implicating urinary TNF in these early renal changes during diabetes. The finding that TNF receptors localize to the apical portion of DT cells further supports the theory that urinary TNF regulates renal function during diabetes. Although urinary TNF appears to be a critical mediator of diabetic nephropathy, the source of urinary TNF remains to be elucidated. Nakamura et al. (26) demonstrate increased TNF mRNA in glomeruli during diabetes. PT cells are also capable of producing TNF (19, 20). TNF secretion from PT segments could subsequently affect DT function in a paracrine manner. TNF could also be produced in DT cells and act on nearby DT cells in an autocrine fashion. PT cells exhibited enhanced TNF mRNA and protein expression after 10 days of diabetes in preliminary experiments in our laboratory, whereas TNF mRNA and protein expression in DT cells was unchanged (unpublished observations). These findings implicate the PT as an important source of urinary TNF and a paracrine regulator of DT function during diabetes. We propose that therapies targeted at reducing PT TNF production should decrease urinary TNF excretion and replicate the therapeutic effects of TNFR:Fc. Pentoxifylline, an agent that inhibits TNF synthesis, may be effective in this regard.

Our results obtained using an animal model of type I diabetes correlate well with clinical observations of Na retention (29, 45) and renal hypertrophy (3, 21) in diabetic patients before the onset of microalbuminuria. Navarro et al. (28) implicate TNF in the development of nephropathy in diabetic patients by demonstrating that the TNF inhibitor pentoxifylline reduces albuminuria. Pentoxifylline also decreases microalbuminuria and overt proteinuria in type II diabetic patients (17). The presence of urinary albumin is the earliest clinical marker of renal dysfunction in diabetic patients (24). Our finding that urinary TNF excretion was significantly increased early in diabetes before the development of microalbuminuria suggests that TNF may be a useful marker of renal dysfunction during diabetes. Elevated urinary TNF may be a clinically relevant marker for early detection of nephropathy on the basis of evidence we provide that TNF participates in renal hypertrophy, which is a predictor of the severity of renal dysfunction in diabetic patients (3).

In summary, these findings provide novel information regarding the role of TNF in early diabetic nephropathy by examining the effects of TNF on isolated kidney cells and utilizing a specific TNF inhibitor (TNFR:Fc) in vivo. We demonstrate that TNF is a critical factor underlying the early pathological changes during diabetic nephropathy, including Na retention and renal hypertrophy. Urinary TNF excretion is increased during diabetes, and reduction of urinary TNF by administration of TNFR:Fc prevents Na retention and hypertrophy. On the basis of these findings, urinary TNF excretion is likely a valuable diagnostic marker for renal dysfunction during the early stages of diabetes. Our finding that increased urine TNF excretion precedes proteinuria is particularly important, because proteinuria is the earliest clinical marker of renal dysfunction during diabetes. Furthermore, inhibition of TNF may represent a novel therapeutic target for preventing the progression of diabetic nephropathy.

We thank Dr. B. Stanton [Dartmouth Cystic Fibrosis Cell Biology Cell Culture Core (STANTO97RO)] for scientific and technical sup-
port; we also thank K. Picha (Immunex) for helpful discussions and for providing TNFR:Fc.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-07301 and DK-57673 and the Joseph Shankman Award from the National Kidney Foundation of Massachusetts, Rhode Island, New Hampshire, and Vermont. Additional support was provided through the Hitchcock Foundation and by the Albert J. Ryan Foundation.

REFERENCES


