Tumor necrosis factor receptors 1 and 2 are associated with early glomerular lesions in type 2 diabetes

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Elevated serum tumor necrosis factor receptor 1 (TNFR1) and 2 (TNFR2) concentrations are strongly associated with increased risk of end-stage renal disease in type 2 diabetes. However, little is known about the early glomerular structural lesions that develop in patients when these markers are elevated. Here, we examined the relationships between TNFRs and glomerular structure in 83 American Indians with type 2 diabetes. Serum TNFRs and glomerular filtration rate (GFR, iothalamate) were measured during a research exam performed within a median of 0.9 months from a percutaneous kidney biopsy. Associations of TNFRs with glomerular structural variables were quantified by Spearman's correlations and by multivariable linear regression after adjustment for age, gender, diabetes duration, hemoglobin A1c, body mass index, and mean arterial pressure. The baseline mean age was 46 years, median GFR 130 ml/min, median albumin/creatinine ratio 26 mg/g, median TNFR1 1500 pg/ml, and median TNFR2 3284 pg/ml. After multivariable adjustment, TNFR1 and TNFR2 significantly correlated inversely with the percentage of endothelial cell fenestration and the total filtration surface per glomerulus. There were significant positive correlations with mesangial fractional volume, glomerular basement membrane width, podocyte foot process width, and percentage of global glomerular sclerosis. Thus, TNFRs may be involved in the pathogenesis of early glomerular lesions in diabetic nephropathy.

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Serum tumor necrosis factor receptor 1 (TNFR1) and receptor 2 (TNFR2) concentrations are strong independent predictors of renal function decline leading to end-stage renal disease (ESRD) in Caucasians and American Indians with diabetes.[1–4]

Although *in vitro*-activated TNFR1 induces tissue injury through proinflammatory signals and/or cell death and TNFR2 promotes cell migration, regeneration, and proliferation, and regulates TNFR1-induced apoptosis,[5] very little is known about the early glomerular structural lesions in kidneys that develop in humans when these markers are elevated. Further, as TNFα binds to the TNFRs, it is not known whether these early glomerular lesions are associated with the serum concentration of TNFα or with the TNFRs. In one small study, TNFR1, but not TNFR2, was associated with higher mesangial fractional volume in 22 persons with type 2 diabetes.[6]

In the present study, we examined the relationships between serum concentrations of TNFα, TNFR1, and TNFR2 and glomerular lesions in American Indians with type 2 diabetes and normal or elevated renal function. The glomerular morphometric data were obtained from a kidney biopsy performed at the end of a 6-year randomized clinical trial that evaluated the renoprotective efficacy of the angiotensin receptor blocker losartan in diabetic nephropathy.[7] The TNF markers were measured in serum obtained at a research examination coincident with the biopsy. The TNF markers that demonstrated univariate associations with glomerular structural lesions were further confirmed using multivariable models.

RESULTS

Clinical and demographic characteristics of the study participants are shown in Table 1. The study included 83 participants with type 2 diabetes (63 female, 20 male), with a mean age of 46 ± 10 years. Forty-three (52%) participants had an albumin-to-creatinine ratio (ACR) <30 mg/g, 24 (29%) had moderate albuminuria (30 to 299 mg/g), and 16 (19%) had severe albuminuria (⩾300 mg/g). Seventy-two (86%)

Table 1 | Characteristics of 83 participants with type 2 diabetes

Clinical characteristics	
Measured markers	
TNFR1 (pg/ml)	1500 (1205–1960)
TNFR2 (pg/ml)	3283 (2670–4151)
TNFα (pg/ml)[a]	4.1 (2.9–5.7)
Other characteristics	
Age (years)[b]	46.3 ± 10.1
Diabetes duration (years)	14.1 (11.7–20.3)
Body mass index (kg/m^2)	34.2 (29.7–40.0)
A1c (%)	9.2 (7.6–11.2)
Systolic blood pressure (mmHg)	124 (111–132)
Diastolic blood pressure (mmHg)	77 (70–84)
Mean arterial pressure (mmHg)	93 (85–99)
Serum creatinine (mg/dL)	0.67 (0.61–0.80)
ACR (mg/g)	26 (12–127)
mGFR (ml/min)	130 (107–174)
mGFR (ml/min per 1.73 m^2)	119 (94–155)
Hypertension treatment (%)[c]	45
Diabetes treatment (%)	90
Lipid-lowering treatment (%)	30

Abbreviations: ACE, angiotensin converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; A1c, hemoglobin A1c; mGFR, iothalamate glomerular filtration rate; TNFα, tumor necrosis factor α; TNFR, tumor necrosis factor receptor. Values are medians (25th and 75th percentiles).
[a]$n = 74$.
[b]Mean ± standard deviation.
[c]$n = 30$ on ACE and 6 on ARB.

participants had measured GFR (mGFR, iothalamate) above 90 ml/min and 81 (98%) had mGFR above 60 ml/min. When mGFR was standardized to body surface area, 66 (79%) participants had mGFR above 90 ml/min per 1.73 m^2 and 78 (94%) had mGFR above 60 ml/min per 1.73 m^2. Hyperfiltration, defined by an mGFR $\geqslant$ 154 ml/min, a value two standard deviations above the mean mGFR for Pima Indians with normal glucose tolerance, was present in 29 individuals (35%).

Serum concentrations of free TNFα and the TNFRs were measured in samples collected at a research examination closest to the kidney biopsy (median of 0.9 months apart, interquartile range = 0.8–1.9 months). Accordingly, 70 participants (84%) were still enrolled in the clinical trial. Thirty-nine (47%) of the participants were assigned to the placebo group and 44 (53%) were assigned to the losartan treatment group during the clinical trial. Table 2 shows the distribution of measured biomarkers and other clinical characteristics at that research examination by the 25th and 75th percentiles of TNFR1 and TNFR2. The mGFR was lower (but not significantly so for TNFR2) and ACR was higher with higher concentrations of either TNFR. Enrollment in the treatment arm of the clinical trial was more common among those in the lower percentile groupings of TNFR1 and TNFR2, but not significantly so. Several glomerular structural variables were significantly associated with percentiles of TNFR1 and TNFR2, as shown in Table 3. Mesangial fractional volume and podocyte foot process width were higher with higher TNFR1 and TNFR2 concentrations, whereas total filtration surface per glomerulus and percentage of the capillary endothelial cell surface covered with normal fenestrations were lower.

TNFR1 and TNFR2 correlated positively with each other ($r = 0.84$, $P < 0.001$) and with ACR ($r = 0.36$ and 0.37, respectively; $P < 0.001$ for each correlation), and inversely with mGFR ($r = -0.35$, $P = 0.001$; $r = -0.28$, $P = 0.01$) (Table 4). Neither TNFR correlated significantly with TNFα. Higher TNFR1 and TNFR2 correlated inversely with the percentage of normally fenestrated endothelium ($r = -0.42$ and -0.43; $P < 0.001$ for each correlation), total filtration surface per glomerulus ($r = -0.27$, $P = 0.01$; $r = -0.29$, $P = 0.007$), and filtration slit frequency ($r = -0.24$, $P = 0.03$; $r = -0.29$, $P = 0.008$), and positively with mesangial fractional volume ($r = 0.36$ and 0.38, $P < 0.001$ for both correlations), glomerular basement membrane width ($r = 0.23$, $P = 0.04$; $r = 0.26$, $P = 0.02$), and podocyte foot process width ($r = 0.29$, $P = 0.007$; $r = 0.31$, $P = 0.004$). TNFR1, but not TNFR2, correlated positively with percent global glomerular sclerosis ($r = 0.25$, $P = 0.02$) and inversely with the number of podocytes per glomerulus ($r = -0.23$, $P = 0.04$). TNFα correlated positively with A1c ($r = 0.27$, $P = 0.02$) and mean arterial pressure ($r = 0.23$, $P = 0.048$), but it had no significant univariate correlations with any glomerular variables.

Given the significant univariate associations of the TNFRs with glomerular lesions, these relationships were then examined after adjusting for age, sex, diabetes duration, A1c, body mass index, and mean arterial pressure (Model 1). Both TNFRs remained significantly and inversely correlated with the total filtration surface per glomerulus (TNFR1 partial $r = -0.25$, $P = 0.03$; TNFR2 partial $r = -0.28$, $P = 0.009$) and the percentage of normally fenestrated endothelium (TNFR1 partial $r = -0.41$, $P = 0.001$; TNFR2 partial $r = -0.37$, $P = 0.0006$) and positively with mesangial fractional volume (TNFR1 partial $r = 0.44$, $P < 0.001$; TNFR2 partial $r = 0.38$, $P = 0.0005$), glomerular basement membrane width (TNFR1 partial $r = 0.34$, $P = 0.002$; TNFR2 partial $r = 0.30$, $P = 0.006$), percent global glomerular sclerosis (TNFR1 partial $r = 0.22$, $P = 0.04$; TNFR2 partial $r = 0.23$, $P = 0.04$), and podocyte foot process width (TNFR1 partial $r = 0.30$, $P = 0.006$; TNFR2 partial $r = 0.25$, $P = 0.02$). Figure 1 shows the strongest of these correlations. The associations between TNFRs and morphometric variables remained unchanged after including treatment assignment from the clinical trial in the multivariable linear regression models (Model 2, Figure 2). When interaction terms between the TNFRs and treatment assignment were added to these models, most were not significant, indicating that the relationship between the TNFRs and most morphometric variables was not modified by treatment. Treatment assignment did modify the relationship between each TNFR and foot process width ($P = 0.02$ for TNFR1, $P = 0.02$ for TNFR2) and filtration slit frequency ($P = 0.001$ for TNFR1, $P = 0.001$ for TNFR2).

Adding ACR and mGFR to the regression models (Model 3) reduced the associations between TNFRs and glomerular lesions, but associations remained significant for mesangial fractional volume (TNFR1 partial $r = 0.27$,