

Remodeling of renal interstitial and tubular lesions in pancreas transplant recipients

P Fioretto¹, DER Sutherland², B Najafian³ and M Mauer³

¹Department of Medical and Surgical Sciences, University of Padova Medical School, Padova, Italy; ²Department of Surgery, University of Minnesota Medical School, Minneapolis, Minnesota, USA and ³Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota, USA

Tubular atrophy and interstitial fibrosis, important in progression of renal diseases, including diabetic (D) and cyclosporine-induced (CSA) nephropathy, have been considered irreversible. Normoglycemia for 10 years following pancreas transplantation alone (PTA) reversed D glomerulopathy lesions. This study quantified tubular, interstitial, and arteriolar parameters in PTA recipients. Kidney function studies and biopsies were performed in eight non-uremic type I D patients (pts) at 5 and 10 years after PTA. Renal biopsies were analyzed by morphometric analysis. All pts were normoglycemic and insulin independent and received CSA during the study. Cortical interstitial volume fraction was increased at 5 years (0.31 ± 0.07 vs normal 0.15 ± 0.02 , $P < 0.01$) and decreased at 10 years post-PTA (0.23 ± 0.03 , $P < 0.02$ vs 5 years). There was a reduction in the volume fraction of interstitial collagen and cells per cortical tissue, measured using electron microscopy, from 5 (0.126 ± 0.061 and 0.103 ± 0.026 , respectively) to 10 years (0.079 ± 0.031 , $P < 0.05$, and 0.074 ± 0.018 , $P < 0.05$, respectively). The volume fraction of tubules which were atrophic (AT) was abnormal at 5 years (0.160 ± 0.090) and decreased from 5 to 10 years (0.044 ± 0.034 , $P < 0.02$), apparently due to AT reabsorption. The index of arteriolar hyalinosis did not change during the study (1.30 ± 0.22 and 1.34 ± 0.33 at 5 and 10 years, respectively, nonsignificant). This study demonstrates, for the first time in humans, that interstitial expansion is reversible and that atrophic tubules can be reabsorbed. In contrast, there was no improvement in the arteriolar lesions. Whether this is due to long-term normoglycemia, reduction of CSA dose or other mechanisms is unclear.

Kidney International advance online publication 22 February 2006; doi:10.1038/sj.ki.5000153;

KEYWORDS: tubular atrophy; interstitial fibrosis; diabetic nephropathy; cyclosporine nephrotoxicity; morphometric analysis; pancreas transplantation

Correspondence: P Fioretto, Department of Medical and Surgical Sciences, University of Padova, Via Giustiniani no. 2, Padova 35128, Italy.
E-mail: paola.fioretto@unipd.it

Received 24 November 2004; revised 25 August 2005; accepted 6 October 2005

Interstitial fibrosis is considered critical to the progressive loss of renal function in different human glomerular diseases.¹⁻⁴ An increase in the relative volume of the cortical interstitium is highly correlated with tubular lesions, especially tubular atrophy; thus, the term tubulo-interstitial lesions is frequently used. The expansion of the interstitial space in chronic renal diseases is mainly due to increased extracellular matrix, although increased cellularity (fibroblasts, macrophages, and lymphocytes) may also contribute.⁵⁻⁷ This accumulation of interstitial extracellular matrix, termed interstitial fibrosis, has generally been considered to be irreversible. Recent studies in experimental animals, however, suggest that the increase in the fraction of the cortical volume occupied by the interstitium ($V_v(\text{Int}/\text{cortex})$), consequent to short-term ureteral obstruction, can improve upon removal of the obstruction,⁸ and that the rate of this interstitial remodeling can be accelerated by angiotensin-converting enzyme inhibitor therapy.⁹ In subtotally nephrectomized rats, high-dose angiotensin-converting enzyme inhibitor treatment induces partial reversal of interstitial and glomerular lesions.¹⁰

Interstitial expansion in man can be influenced by treatment. Two years of angiotensin-converting enzyme inhibitor therapy halted the increase in $V_v(\text{Int}/\text{cortex})$ in type II diabetic (D) patients (pts) with nephropathy, while there was a further increase in this parameter in the untreated group.¹¹ Reversal of interstitial expansion in humans, however, has not been demonstrated; thus, as recently discussed,¹² whether the results achieved in rats are applicable to chronic kidney disease in humans is currently unknown. We have previously reported that mesangial expansion as well as glomerular (GBM) and tubular basement membrane (TBM) thickening can be reversed in type I D pts' native kidneys following successful pancreas transplantation alone (PTA),¹³ demonstrating that these renal structures can undergo substantial architectural remodeling, with net removal of glomerular extracellular matrix material. Increased $V_v(\text{Int}/\text{cortex})$, a common finding in long-standing diabetes,¹⁴⁻¹⁸ was present in all the pts in this study before PTA.¹⁹ These pts, in addition, received cyclosporine, which is known to cause interstitial fibrosis.²⁰ The present study explored whether remodeling and healing can also

occur in the renal interstitium and tubules in these PTA recipients.

RESULTS

Cyclosporine (CSA) dose decreased from 8.5 ± 3.2 mg/kg/day in the first year after PTA to 5.0 ± 1.9 mg/kg/day at 5 years ($P < 0.01$) and to 3.8 ± 1.5 mg/kg/day at 10 years after PTA ($P < 0.05$ vs 5 years). CSA blood levels decreased from 193 ± 64 μ g/l in the first year to 102 ± 40 μ g/l at 5 years ($P < 0.005$) and 71 ± 24 μ g/l at 10 years after PTA ($P < 0.05$ vs 5 years). Significant correlations were observed between CSA daily dose and CSA blood levels ($P < 0.01$). Also, as previously shown, there were significant correlations between CSA dose and blood levels in the first PTA year and the reduction in creatinine clearance.¹⁹

Renal structure

Light microscopy. Cortical interstitial volume fraction ($V_v(\text{Int}/\text{cortex})$) was 0.31 ± 0.07 at 5 years and significantly decreased at 10 years post-PTA (0.23 ± 0.03 , $P < 0.02$) (Figure 1a). These quantitative findings were associated with a decrease from 5 to 10 years in the subjectively estimated extent of interstitial periodic acid-Schiff (PAS)-positive staining in these biopsies (Figures 2a and b). Similarly, the fractional volume of ATs ($V_v(\text{AT}/\text{TT})$) decreased from 0.160 ± 0.090 at 5 years to 0.044 ± 0.034 at 10 years ($P < 0.02$) (Figure 1b).

Average tubular profile areas were similar at 5 years (1310 ± 276 μm^2) and at 10 years (1430 ± 353 μm^2 , nonsignificant (NS)), as were average distal tubular profile areas (597 ± 95 and 571 ± 207 μm^2 at 5 and 10 years, respectively, NS).

Arteriolar hyalinosis score was 1.30 ± 0.23 at 5 years and remained unchanged at 10 years (1.34 ± 0.37 , NS).

The percent of sclerotic glomeruli was $29 \pm 20\%$ at 5 years and $35 \pm 17\%$ at 10 years (NS). All biopsies contained one or more sclerotic glomeruli. Most of the sclerotic glomeruli in the 5- and 10-year biopsies were incompletely surrounded by Bowman's capsule. The percentage of the profile of sclerotic glomeruli which was surrounded by Bowman's capsule

decreased from 5 ($60.4 \pm 30.1\%$) to 10 years ($28.3 \pm 11.1\%$, $P < 0.03$). Where Bowman's capsule was partially or completely lost from the profile, direct contact of interstitial cells with the tuft material of sclerotic glomeruli was regularly observed.

Two of eight biopsies at 5 years and seven of eight at 10 years ($P < 0.001$) had one or more sclerotic glomeruli without adjacent AT. Figure 2c illustrates this phenomenon. Also, of note, some sclerotic glomeruli appeared to be losing PAS-positive basement membrane material (Figure 2b and c).

The changes in albumin excretion rate (AER) from 5 to 10 years were not significantly correlated with the changes in $V_v(\text{Int}/\text{cortex})$ ($r = 0.67$, $P = 0.07$) and $V_v(\text{AT}/\text{TT})$ ($r = 0.62$, $P = 0.10$) over the same time interval. Also, the changes in AER from baseline (before-PTA) to 10 years were not correlated with the changes in the structural parameters from the fifth to the 10th year.

Electron microscopy. The volume fractions of the four interstitial components, $V_v(\text{Cell}/\text{int})$, $V_v(\text{Col}/\text{int})$, $V_v(\text{Cap}/\text{int})$, and $V_v(\text{Space}/\text{int})$, were not significantly different between the 5 and 10 years post-PTA biopsies, although there was a trend towards an increase in $V_v(\text{Cap}/\text{int})$ from the fifth to the 10th year ($P = 0.096$; Table 1). The volume fraction of the cellular and collagen interstitial components per cortical tissue, in contrast, decreased from the fifth to the 10th year post-proximal tubular (PT) profile (Table 1).

DISCUSSION

This study demonstrates, for the first time in human renal disease, that interstitial expansion is reversible and that atrophic tubules (ATs) can be reabsorbed. We documented architectural remodeling in glomerular and tubular extracellular matrix structures in long-standing type I D pts rendered normoglycemic for 10 years by successful PTA.¹³ This was not demonstrable in the 5-year biopsies.²¹ However, this was documented by electron microscopic morphometric analysis as reductions between the fifth and the 10th year after PTA in glomerular basement membrane and TBM thickening, and in mesangial matrix expansion, and was manifest, by light microscopy, as reversal of D glomerular lesions, including the disappearance of Kimmelstiel-Wilson nodules.¹³

The improvement in interstitial expansion between the fifth and 10th year following PTA may reflect similar phenomena. This improvement apparently results from interstitial extracellular matrix removal exceeding extracellular matrix production. The reduction in interstitial fractional volume was not associated with a statistically significant change in interstitial composition. However, considered in the context of cortical tissue, there was a significant decrease in total amount of interstitial collagen and cellular material (Table 1). This was consistent with a subjective decrease in light microscopic interstitial PAS-positive staining (Figures 2a and b). A less likely explanation for the reduction in interstitial fractional volume is that renal

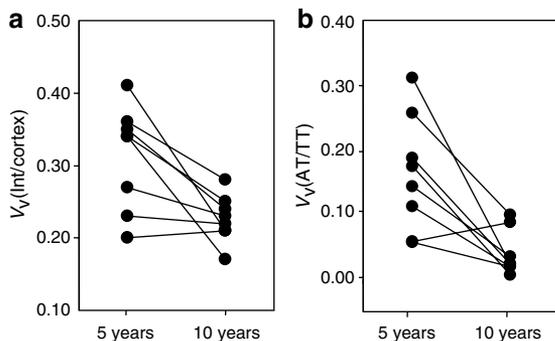


Figure 1 | Cortical interstitial volume fraction ($V_v(\text{Int}/\text{cortex})$) and fractional volume of ATs ($V_v(\text{AT}/\text{TT})$) at 5 and 10 years after pancreas transplantation. Data for individual pts are connected by lines.

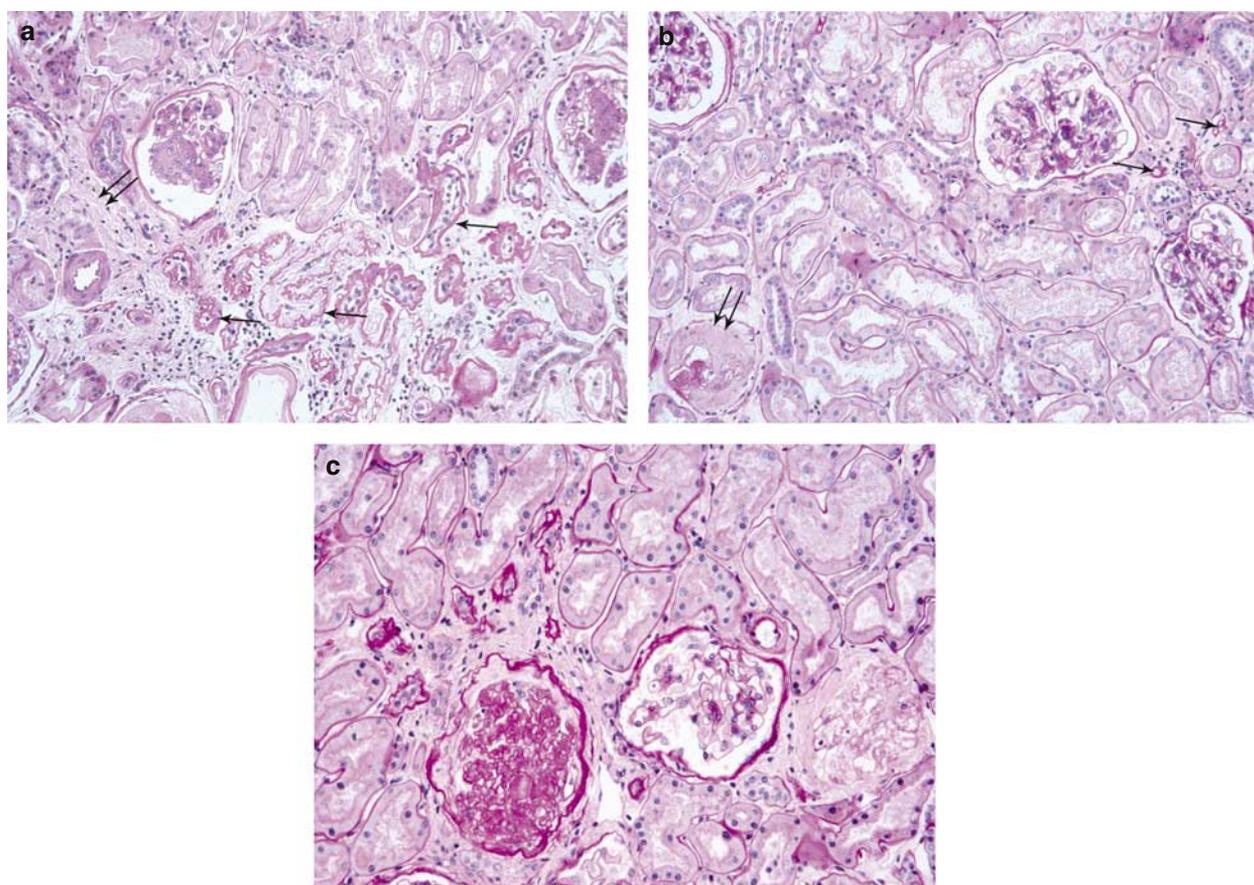


Figure 2 | Photomicrographs of renal biopsy specimens obtained 5 and 10 years after PTA from the same patient (periodic acid-Schiff). Panel a illustrates the 5-year post-PTA renal biopsy specimen. There are areas of advanced interstitial fibrosis (double arrows) and tubular atrophy (single arrows). Note the presence of diffuse and nodular D glomerulopathy. Panel b illustrates the 10-year post-PTA biopsy specimen. There is marked improvement in the interstitial fibrosis compared to panel a. Only a few TBM remnants of ATs remain (single arrow). There is a globally sclerotic glomerulus surrounded by normal tubules (double arrow). The remaining glomeruli show almost complete resolution of D glomerular changes compared to panel a. Panel c: This photomicrograph of the 10-year post-PTA biopsy illustrates three glomeruli: the central glomerulus is nearly normal; the globally sclerotic glomerulus on the left contains intensely PAS-positive staining basement membrane and mesangial matrix materials, and is surrounded by intact Bowman's capsule, ATs, and interstitial fibrosis. The glomerulus on the right is also globally sclerotic, but with much less PAS positivity in the extracellular matrix staining and nearly absent Bowman's capsule. The tubules surrounding this pale sclerotic glomerulus are normal, but for a small TBM remnant PAS, original magnification $\times 150$.

Table 1 | Interstitial composition by electron microscopy at 5 and 10 years after pancreas transplantation

	5 years post-PTA	10 years post-PTA	P-value
$V_v(\text{Cell/int})$	0.355 ± 0.152	0.329 ± 0.070	0.554
$V_v(\text{Cell/cortex})$	0.103 ± 0.026	0.074 ± 0.02	0.046
$V_v(\text{Coll/int})$	0.384 ± 0.132	0.371 ± 0.098	0.715
$V_v(\text{Coll/cortex})$	0.127 ± 0.061	0.079 ± 0.032	0.049
$V_v(\text{Cap/int})$	0.199 ± 0.056	0.248 ± 0.063	0.096
$V_v(\text{Cap/cortex})$	0.063 ± 0.025	0.055 ± 0.012	0.376
$V_v(\text{Space/int})$	0.068 ± 0.040	0.052 ± 0.027	0.453
$V_v(\text{Space/cortex})$	0.023 ± 0.016	0.013 ± 0.009	0.208

Data are presented as mean \pm s.d.

$V_v(\text{Cell/int})$, $V_v(\text{Coll/int})$, $V_v(\text{Cap/int})$ and $V_v(\text{Space/int})$ =fractional volume of cells, collagen, capillaries and space per interstitium.

$V_v(\text{Cell/cortex})$, $V_v(\text{Coll/cortex})$, $V_v(\text{Cap/cortex})$ and $V_v(\text{Space/cortex})$ =fractional volume of cells, collagen, capillaries and space per cortical tissue.

size increased between the fifth and the 10th year post-PTA. However, normalization of blood glucose levels should lead to a reduction in kidney size rather than an increase.²² Since kidney size data were not available, we measured mean

proximal and distal tubular profile areas. Tubules make up approximately 85% of cortical volume and renal hypertrophy is regularly associated with tubular enlargement. The nearly identical tubular profile areas at 5 and 10 post-PTA years is strong evidence that renal hypertrophy did not occur over that time. These findings indicate that the reduction in interstitial fractional volume between the fifth and 10th post-PTA year is consequent to the disappearance of interstitial tissue, especially collagen and cellular components, both of which have been shown to contribute to the increase in interstitial fractional volume in D pts.²³

There was also a reduction between the fifth and 10th post-PTA year in the fractional volume of cortical AT. This appeared, at least in part, to be due to the disappearance of AT, as demonstrated by studying those tubular profiles surrounding sclerotic glomeruli. This reabsorption process probably involves additional mechanisms, likely including the recruitment of circulating mononuclear leukocytes,⁵⁻⁷ resulting in complete removal of the extracellular matrix and

cellular components of these AT, rather than remodeling towards normal. In contrast, when the non-atrophic proximal tubules were studied, we observed reversal of the thickening of TBMs.¹³ Much more would need to be known about these processes before it could be understood how these systems are regulated, whereby tubules with TBM thickening undergo TBM remodeling while tubules with more advanced injury, particularly those related to sclerotic glomeruli, are completely removed.

The potential significance of these interstitial and tubular findings derives from the strong relationship between the severity of tubular and interstitial injury and clinical progression of established renal disease,¹⁻⁴ including D nephropathy.^{17,18} It was a major concern, therefore, that the interstitial fibrosis, already present in all pts before transplantation, and thus consequent to diabetes,^{15,18} had worsened at 5 years after PTA, despite cure of the D state. This worsening was probably due to the administration of CSA following PTA, since the severity of interstitial expansion between the baseline and the 5 years biopsy was highly correlated with the dose and blood levels of CSA during the first year after PTA.¹⁹ There was also an increase in $V_v(\text{AT}/\text{TT})$ and the percentage of globally sclerotic glomeruli between the baseline and 5-year biopsies.¹⁹

Reductions in CSA dose over time or prolonged normoglycemia or both could have contributed to the findings of interstitial structural improvement in our studies. Our study design, however, did not allow dissection between these or other possible hypotheses. For example, we documented a reduction at 5 and 10 years in AER in the PTA recipients with elevated baseline AER values, and proteinuria and tubulointerstitial injury have been pathogenetically linked.²⁴⁻²⁶ The changes in AER in our study tended to correlate with the changes in interstitial and tubular parameters. Thus, given the small number of pts, the lack of statistically significant correlations between the magnitude of AER reduction and the changes in structural parameters from the fifth to the 10th year does not rule out this possibility.

Nonetheless, whatever the mechanisms, these studies document the potential for reversal of interstitial fibrosis in humans. This is most unlikely to occur spontaneously, as in a group of pts with persistent type I diabetes $V_v(\text{Int}/\text{cortex})$ and $V_v(\text{AT}/\text{TT})$, increased at first observation, did not change over 5 years of follow-up.¹⁹

The percent of glomeruli that were globally sclerotic did not change between the fifth and the 10th year. This is consistent with the sclerotic glomeruli not being reabsorbed, taking longer to be removed than their associated AT, or continuing to develop at about the same rate as they are removed. We favor the second hypothesis since some sclerotic glomeruli appeared to be losing PAS-positive basement membrane material (Figure 2c). However, given the continuous gradation of staining of sclerotic glomeruli, this was not quantifiable. Moreover, many sclerotic glomeruli had lost at least part of Bowman's capsule. This Bowman's capsular loss

promoted direct access of interstitial cells to glomerular tuft material, likely facilitating glomerular reabsorption.

Arteriolar hyalinosis lesions, common to both D nephropathy²⁷ and CSA nephrotoxicity,^{20,28} did not change between the fifth and 10th post-PTA years. Thus, once established, these lesions may not be reversible and, previously linked to glomerular sclerosis,²⁷ may be important in clinical progression of both disorders.

In conclusion, whether secondary to long-term normoglycemia, reduction in CSA dose, or other factors, this study demonstrates that the human kidney has the mechanisms necessary to heal interstitial fibrosis, remove AT, and undergo architectural remodeling towards normal. Given the importance of tubular and interstitial lesions to the progression of chronic renal diseases,^{1-4,18} understanding of the cellular regulation of these healing phenomena could lead to new therapeutic approaches.

MATERIALS AND METHODS

Pts and study design

Type I D pts who were recipients of successful PTA and were insulin independent and euglycemic for 10 years after PTA were eligible for this study. These pts, in addition, had to have a kidney biopsy performed before and 5 and 10 years after PTA.¹³ Of the original cohort of 13 pts studied at 5 years after PTA,²¹ two developed end-stage renal disease between the fifth and 10th post-PTA years; these two pts had the most advanced renal structural changes and renal dysfunction at the time of PTA. Eight pts meeting these criteria were included. Four had received a cadaveric pancreas and four a segmental pancreas graft from living related donors. After induction therapy, all pts received immunosuppression with prednisone, azathioprine, and cyclosporine (CSA) throughout the 10 years of the study. These studies were approved by the Committee for the Use of Human Subjects in Research of the University of Minnesota. All pts gave written informed consent before each evaluation. Measures of renal function, metabolic parameters, and percutaneous renal biopsy were obtained before and 5 and 10 years after PTA. The results of the baseline, 5 and 10 years follow-up clinical data, and of D glomerulopathy and TBM lesions have been reported previously.¹³ Briefly, pts (2M/6F) were 33 ± 3 years old, with D duration of 22 ± 5 years at the time of PTA. Glycosylated hemoglobin was normal at 5 and 10 years (5.3 ± 0.4 and $5.5 \pm 0.7\%$, respectively). Fasting blood glucose levels were 89.9 ± 4.45 and 95.9 ± 9.2 mg/dl at 5 and 10 years, respectively; fasting insulin levels were 23.3 ± 19.9 and 19.9 ± 6.8 $\mu\text{U}/\text{ml}$ at 5 and 10 years. Fasting plasma C-peptide data in insulin-independent long-term successful pancreas transplant recipients at our institution are 3.47 ± 0.36 (mean \pm s.e.) in recipients of whole pancreas and 1.95 ± 0.37 ng/ml in segmental graft recipients, and 1.25 ± 0.11 in normal controls, as reported previously.²⁹ Creatinine clearance decreased from 108 ± 20 to 65 ± 12 ml/min/1.73 m² at 1 year post-PTA ($P < 0.001$) and did not change significantly thereafter (74 ± 16 and 74 ± 14 ml/min/1.73 m² at 5 and 10 years, respectively).¹³ AER was 103 (7-1276) mg/24 h before PTA and did not significantly change at 5 years (30 (2-155) mg/24 h, $P = 0.07$ vs baseline) and 10 years (20 (6-176) mg/24 h, NS vs baseline and 5 years) although there was a trend towards reduction. AER was significantly reduced when only recipients with elevated baseline AER values were considered.¹³ The mean blood pressure also did not statistically significantly change

(88 ± 5 , 92 ± 8 and 97 ± 12 mmHg at baseline, 5, and 10 years, respectively); however, the number of pts receiving antihypertensive therapy increased from 2 before PTA to 5 at 5 and 10 years. No pt received angiotensin-converting enzyme inhibitor or lipid-lowering agents during the study.¹³

Here we focus on the 5- and 10-year results related to structural studies of the renal interstitium, tubules, and arterioles.

Procedures

All pts spent 1 week in the General Clinical Research Center at the University of Minnesota for pre-PTA evaluation and several days for each follow-up evaluation. During each hospitalization, pts underwent multiple 24-h urine collections (at least three) for measurements of creatinine clearance by the Jaffé reaction (normal range: 90-130 ml/min/1.73 m²) and urinary AER by nephelometry (normal values: <22 mg/24 h). Blood pressure was measured repeatedly by the General Clinical Research Center nursing staff. Glycosylated hemoglobin was measured by high-performance liquid chromatography (BioRad Diamat, BioRad Laboratories, Hercules, CA, USA) (normal range: 4.0-6.1%), and insulin levels by radioimmunoassay. CSA blood levels were measured by high-performance liquid chromatography on blood samples drawn 12 h after CSA administration.

Renal biopsy studies

Light microscopy. Tissue for light microscopy was fixed in Zenker's solution, embedded in paraffin, cut in 2-3 μ m sections, and stained with PAS stain. All morphometric measurements and semiquantitative estimates were blindly performed.

$V_v(\text{Int}/\text{cortex})$, the fraction of the renal cortex which is interstitium, was estimated using a projection microscope at $\times 300$, as described previously.¹⁹ All available cortical tissue was measured. Points falling on the interstitium, defined as the space outside Bowman's capsule, TBM, and vessels larger than one tubular diameter, and total number of points overlying the cortical tissue were counted using a 1:4 grid with a distance between fine points of 13 mm (normal value: 0.15 ± 0.02).

Percent sclerosed glomeruli was determined when at least 20 glomeruli were available for study²⁷ (normal value: <10%). All but two of the 24 biopsies examined were adequate for this measurement. In order to explore whether the sclerosed glomeruli could be undergoing reabsorption, a semiquantitative estimate of the percentage of the profile of each sclerosed glomerulus surrounded by intact Bowman's capsule was performed.

Tubular atrophy was defined by the presence of thickened or reduplicated TBM surrounding tubules of reduced diameters, containing shortened or flat tubular epithelial cells; in the absence of thickened TBM, atrophy was defined as a tubular diameter reduced to less than 50% of normal, as determined by comparison with profiles of adjacent tubules surrounded by TBM and with the presence of luminal space in the tubular profile.²⁸ Fractional volume of AT, the proportion of the volume of total cortical tubules (TT) composed of AT ($V_v(\text{AT}/\text{TT})$), was estimated by point counting using a projection microscope at $\times 300$. All available cortical tissue was measured. A 1:9 grid with a distance between fine points of 10 mm was used. Points falling on AT were divided by the total number of points falling on all cortical tubules.

The average tubular profile area of non-AT was estimated on sections with adequate cortical tissue on the 5- and 10-year post-PTA biopsies. Sections were viewed using a projection microscope at

$\times 300$. A staggered cycloid grid with cycloid length per point (l/p) = 100 μ m and area per point (a/p) = 714 μ m² was superimposed on the projected images. The short axes of cycloids were aligned parallel to the long axes of tissue sections (vertical axes). Three to six adjacent non-overlapping fields per slide were studied per biopsy, yielding a minimum number of 150 points hitting proximal tubular (PT) profiles and of 150 intercepts between the cycloids and TBM. The surface density of PT per cortex was estimated as described elsewhere.³⁰

The volume density of PT per cortex ($V_v(\text{PT}/\text{cortex})$) was estimated by point counting. The average proximal TPA was estimated using the following equation:³¹

$$\text{TPA} = \frac{4\pi V_v^2(\text{PT}/\text{cortex})}{S_v^2(\text{PT}/\text{cortex})}$$

The same method was used to determine the average distal tubular profile area.

The possibility that partial or complete reabsorption of AT could occur was evaluated as follows. Since sclerosed glomeruli are typically associated with adjacent AT, light microscopic slides were blindly evaluated for the presence of sclerotic glomeruli, which were not accompanied by AT within a distance of one normal tubular diameter from the scarred glomerulus. This analysis was performed on one slide only (serial sections were not available on all biopsies), and therefore we cannot exclude the possibility that residual AT were present adjacent to the sclerotic glomerulus in another section plane.

The index of arteriolar hyalinosis was obtained by subjective estimation of the fraction of each arteriolar wall replaced by hyaline material in one complete light microscopy section, as described previously.³² Then, the following formula was applied:

$$\begin{aligned} \text{Numerator} : & 1 \times n \text{ of arterioles with a score } 0.25 \\ & 2 \times n \text{ of arterioles with a score } 0.25 - 0.50 \\ & 2 \times n \text{ of arterioles with a score } 0.51 - 0.75 \\ & 4 \times n \text{ of arterioles with a score } 0.76 \end{aligned}$$

Denominator: Total number of arterioles counted.

A mean of 32 arterioles (range: 9-110) were evaluated per biopsy.

Electron microscopy. Interstitial composition was evaluated on the 5 and 10 years post-PTA biopsies using stereological methods on images obtained at $\times 7500$ by systematic unbiased sampling through predetermined movements of the EM stage controls, as described previously.²³ During acquisition of the micrographs, areas containing a part of a glomerulus or vessels larger than a tubule diameter were excluded. Thus, sampling was preferentially one tubular diameter or more from these structures. This largely avoided the potential bias of sampling of peri-glomerular fibrosis or vascular adventitia. Two EM blocks per biopsy were studied, with the acquisition of 62 ± 18 micrographs per biopsy. The cortical interstitium was divided into four components: cells (Cell), collagen (Coll), capillaries (Cap), and 'undefined space' (Space). The volume fraction of the cortical interstitium occupied by each interstitial component, $V_v(\text{Cell}/\text{int})$, $V_v(\text{Coll}/\text{int})$, $V_v(\text{Cap}/\text{int})$, and $V_v(\text{Space}/\text{int})$ was estimated by point counting.²³ Volume fractions of these interstitial components were measured using a 1:9 lattice grid with a distance between fine points of 20 mm. Coarse points were used to quantitate the interstitium, while fine points were counted on each of the four interstitial components. The mean number of coarse and fine points counted per biopsy was 535 ± 108 and 1941 ± 398 , respectively. The volume fraction of the cortical interstitium occupied by each component was calculated as $V_v(\text{Comp}/$

Int) = $FPx/(CP \times 9)$. The volume fraction of the cortical tissue occupied by each interstitial component was obtained by multiplying the volume fraction of each compound with $V_v(\text{Int}/\text{cortex})$.

Statistics

Results are expressed as mean \pm 1s.d., except for AER which, not normally distributed, is expressed as the median and range. AER values were logarithmically transformed before analyses. Comparisons of 5- and 10-years follow-up data used the paired two-sided Student's *t*-test. Linear regression analyses were used to evaluate the relationships between CSA dose and plasma levels, and between changes in AER and in structural parameters. The binomial test was used to compare the observed frequencies of biopsies with and without sclerotic glomeruli, which were unaccompanied by AT. Where there was a 0 in one of these categories, a probability value of 1% was assigned to that category and 99% to the other. Statistical significance was set at $P < 0.05$; however, all *P*-values < 0.10 are provided.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (DK13083), the National Center for Research Resources (MO1-KK00400), and by an endowment from the Kroc Research Foundation. During the initial stages of this work, Dr Fioretto was supported by Research Fellowship and Career Development Awards from the Juvenile Diabetes Foundation International. We are grateful to Ms Susan Sisson-Ross for excellent technical assistance, and Dr Maria Luiza A Caramori for help with the statistical analyses. We are indebted to the patients who generously participated in these studies.

REFERENCES

- Risdon RA, Sloper JC, de Wardener HE. Relationship between renal function and histological changes found in renal-biopsy specimens from patients with persistent glomerular nephritis. *Lancet* 1968; **2**: 363-366.
- Schainuck LI, Striker GE, Cutler RE, Benditt EP. Structural-functional correlations in renal disease. *Hum Pathol* 1970; **1**: 631-641.
- D'Amico G. Tubulointerstitium as predictor of progression of glomerular diseases (editorial). *Nephron* 1999; **83**: 289-295.
- Nath KA. Tubulointerstitial changes as a major determinant in the progression of renal damage. *Am J Kidney Dis* 1992; **20**: 1-17.
- Nabokov A, Waldherr R, Ritz E. Demonstration of the proliferation marker Ki-67 in renal biopsies: correlation to clinical findings. *Am J Kidney Dis* 1997; **30**: 87-97.
- Markovic-Lipkovski J, Müller CA, Rislis T et al. Association of glomerular and interstitial mononuclear leukocytes with different forms of glomerulonephritis. *Nephrol Dial Transplant* 1990; **5**: 10-17.
- Hooke DH, Gee DC, Atkins RC. Leukocyte analysis using monoclonal antibodies in human glomerulonephritis. *Kidney Int* 1987; **131**: 964-972.
- Chevalier RL, Kim A, Thornhill BA, Wolstenholme JT. Recovery following relief of unilateral ureteral obstruction in the neonatal rat. *Kidney Int* 1999; **55**: 793-807.
- Koo JW, Kim Y, Rozen S, Mauer M. Enalapril accelerates remodeling of the renal interstitium after release of unilateral ureteral obstruction in rats. *J Nephrol* 2003; **16**: 203-209.
- Adamczak A, Gross M-L, Krtli J et al. Reversal of glomerulosclerosis after high-dose Enalapril treatment in subtotaly nephrectomized rats. *J Am Soc Nephrol* 2003; **14**: 2833-2842.
- Cordonnier DJ, Pinel N, Barro C et al. Expansion of cortical interstitium is limited by converting enzyme inhibition in type 2 diabetic patients with glomerulosclerosis. The Diabiopsies Group. *J Am Soc Nephrol* 1999; **6**: 1253-1263.
- Fogo AB. Regression lines in chronic kidney disease. *J Am Soc Nephrol* 2003; **14**: 2990-2991.
- Fioretto P, Steffes MW, Sutherland DER et al. Reversal of lesions of diabetic nephropathy after pancreas transplantation. *New Engl J Med* 1998; **339**: 69-75.
- Bader R, Bader H, Grund KE et al. Structure and function of the kidney in diabetic glomerulosclerosis. Correlations between morphological and functional parameters. *Pathol Res Pract* 1980; **167**: 204-216.
- Böhle A, Wehrmann M, Bogenschütz O et al. The pathogenesis of chronic renal failure in diabetic nephropathy. Investigation of 488 cases of diabetic glomerulosclerosis. *Pathol Res Pract* 1991; **187**: 251-259.
- Taft JL, Nolan CJ, Yeung SP et al. Clinical and histological correlations of decline in renal function in diabetic patients with proteinuria. *Diabetes* 1994; **43**: 1046-1051.
- Mauer SM, Steffes MW, Ellis EN et al. Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 1984; **74**: 1143-1155.
- Lane PH, Steffes MW, Fioretto P, Mauer SM. Renal interstitial expansion in insulin-dependent diabetes mellitus. *Kidney Int* 1993; **43**: 661-667.
- Fioretto P, Steffes MW, Mihatsch MJ et al. Cyclosporine-associated lesions in native kidneys of diabetic pancreas transplant recipients. *Kidney Int* 1995; **48**: 489-495.
- Myers BD. Cyclosporine nephrotoxicity. *Kidney Int* 1986; **30**: 964-974.
- Fioretto P, Mauer SM, Bilous RW et al. Effects of pancreas transplantation on glomerular structure in insulin-dependent diabetic patients with their own kidneys. *Lancet* 1993; **342**: 1193-1196.
- Tuttle KR, Bruton JL, Perusken MC et al. Effect of strict glycemic control on renal hemodynamic response to aminoacids and renal enlargement in insulin-dependent diabetes mellitus. *N Engl J Med* 1991; **324**: 1626-1632.
- Katz A, Caramori ML, Sisson-Ross S et al. An increase in the cell component of the cortical interstitium antedates interstitial fibrosis in type 1 diabetic patients. *Kidney Int* 2002; **61**: 2058-2066.
- Remuzzi G, Bertani T. Pathophysiology of progressive nephropathies. *N Engl J Med* 1998; **339**: 1448-1456.
- Eddy A. Role of cellular infiltrates in response to proteinuria. *Am J Kidney Dis* 2001; **37**(Suppl 2): S25-S29.
- Remuzzi G. A unifying hypothesis for renal scarring linking protein trafficking to different mediators of injury. *Nephrol Dial Transplant* 2000; **15**(Suppl 6): 58-60.
- Harris RD, Steffes MW, Bilous RW et al. Global glomerular sclerosis and glomerular arteriolar hyalinosis in insulin dependent diabetes. *Kidney Int* 1991; **40**: 107-114.
- Mihatsch MJ, Antonovych T, Bohman S-O et al. Cyclosporin A nephropathy: standardization of the evaluation of kidney biopsies. *Clin Nephrol* 1994; **41**: 23-32.
- Robertson PR. Consequences on β -cell function and reserve after long-term pancreas transplantation. *Diabetes* 2004; **53**: 633-644.
- Baddeley AJ, Gundersen HJG, Cruz-Orive LM. Estimation of surface area from vertical sections. *J Microsc* 1986; **142**: 259-276.
- Najafian B, Mauer M. Estimating length density of tubular structures on thin vertical sections and its application in evaluating intertubular space changes. *FASEB J* 2003; **17**: A1374-A1375.
- Drummond K, Mauer M, International Diabetic Nephropathy Study Group. The early natural history of nephropathy in type 1 diabetes: II. Early renal structural changes in type 1 diabetes. *Diabetes* 2002; **51**: 1580-1587.