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The long-term course of cyclosporine-associated chronic nephropathy

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The long-term course of cyclosporine-associated chronic nephropathy. We evaluated a chronic renal injury in 37 cardiac transplant recipients treated for 12 to 24 months with cyclosporine (CsA). Twenty-four cardiac transplant recipients treated with azathioprine for more than 24 months served as controls. Despite equivalent cardiac performance, GFR in those treated with CsA was depressed, 47 ± 3 versus 94 ± 4 ml/min/1.73 m^2 ($P < 0.001$). CsA therapy was also associated with significant elevation of renal vascular resistance (RVR), proteinuria, arterial hypertension, and impaired intrarenal conversion of inactive prorenin to active renin. Histopathological changes associated with CsA included an obliterative arteriolopathy with deposition of proteinaceous material in necrotic arteriolar walls, and associated tubulointerstitial damage. A minority of glomerbuli exhibited either ischemic collapse or sclerosis. Area perimeter analysis revealed enlargement of the remaining glomeruli with significant expansion of the mesangium. Longitudinal examination over a 48 month period ($N = 15$) during which CsA was reduced in dosage or withdrawn revealed persistent hypofiltration, increasingly elevated RVR and heavier proteinuria. Further histopathological deterioration was observed when renal tissue was sampled a second time in six patients, and three members of the experimental group developed end-stage renal disease. We conclude that continuous CsA therapy for more than 12 months causes a chronic injury to renal microvessels that is rarely reversible and potentially progressive.

Cyclosporine A (CsA) has been used to suppress cardiac allograft rejection at our institution since December 1980, two years after its introduction into clinical medicine as an immunosuppressive agent [1]. Although graft survival was enhanced, it soon became apparent that chronic administration of CsA was associated with the development of a chronic renal injury. Careful study of renal function and structure in patients who had received CsA therapy for 12 months revealed a substantial decline in the glomerular filtration rate (GFR), accompanied by patchy collagenisation of glomeruli and the tubulointerstitial compartment [2, 3].

In the hope that the diminished mortality and improved well-being associated with CsA would be maintained, we have continued to immunosuppress cardiac transplant recipients with this agent, albeit at progressively lower dosages. To elucidate the nature and outcome of the chronic renal injury associated with protracted CsA therapy we have extended our earlier series of observations [2, 3]. We have enlarged both in scope and number measurements of renal function in patients receiving CsA for 12 or 24 months, and attempted to relate these to alterations in renal morphology. We have also subjected a subset of our CsA treated population to a longitudinal examination over a 48 month period. Our cross-sectional observations reveal that at the relatively high dosage used in the initial part of this study, CsA therapy of 12 to 24 months duration leads to a severe injury of renal microvessels. Our longitudinal observations indicate that this chronic microvascular injury is not reversed by either late reduction of dosage or even complete withdrawal of CsA therapy.

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Methods

Patient populations

Routine clinical and laboratory determinations were made at yearly intervals in each of 100 patients who received consecutive cardiac transplants between December 1980 and April 1984, when CsA combined with prednisone was the standard immunosuppressive regimen at our institution. CsA was initiated with an oral loading dose of 17 mg/kg directly before the transplantation and lowered postoperatively to an average dose of 10 mg/kg per 24 h by the end of the first month. Subsequent maintenance CsA therapy was adjusted to achieve trough levels of immunoassayable CsA in serum between 100 and 300 ng/ml (through May 1984) or 50 to 150 ng/ml (June 1984 and thereafter). Control observations were made in 100 patients who received consecutive cardiac transplants between April 1976 and December 1980 when azathioprine combined with prednisone was used to suppress allograft rejection.

Approximately one half of the available members of each group were selected arbitrarily for a detailed examination of renal function using physiological techniques. Of 73 CsA treated recipients surviving for two years beyond transplantation, 37 were subjected to physiologic study after 12 or 24 months of therapy. Forty-seven patients treated under the earlier azathioprine regimen were alive when we commenced our study in February 1983, and 24 of these were selected for physiologic study. No criteria other than patient willingness to participate and the availability of a team of investigators were used in

selecting the subgroups for detailed study. The subgroups did not differ with respect to age (36 ± 1 vs. 38 ± 1 years in CsA vs. azathioprine recipients), sex distribution, or etiology of the original heart disease. Neither was the prevailing serum creatinine level at the time of transplantation different in the two groups, 1.3 ± 0.1 versus 1.4 ± 0.1 mg/dl, respectively.

Physiologic studies

It is routine procedure at our institution to admit cardiac transplant recipients to a clinical research center at yearly intervals for evaluation of cardiac allograft function. This evaluation includes determination of cardiac output according to the Fick method, and measurement of hydraulic pressures in the cardiac chambers, superior vena cava and femoral artery. During the 27 months from February 1, 1983, to May 1985, the selected patients of the two subgroups described above, also underwent a detailed physiologic study of renal function during their annual evaluation. Of the 37 CsA-treated patients selected, 20 had received their cardiac allograft one year previously, while the remaining 17 had been transplanted two years earlier. The 24 members of the azathioprine-treated control population were undergoing their third through fifth annual examination of cardiac allograft function. The study protocol, previously approved by the institutional review board at Stanford University, was described in detail to all patients before admission, and informed consent to perform the study was obtained in each instance. Antihypertensive and diuretic drugs were discontinued for four days before admission in all patients receiving such therapy.

Before transferring patients to the cardiac catheterization laboratory, each was studied according to a urinary clearance technique that has been described in detail elsewhere [2]. In brief, blood was drawn first after the patient had remained supine overnight for estimation of hematocrit level, plasma oncotic pressure and examination of the renin-angiotensin system. Clearances of inulin and paraaminohippuric acid (PAH) were then performed with the subject voiding spontaneously after a diuresis had been established by oral water loading. The GFR was calculated as the urinary clearance of inulin averaged for each of four collection periods, and corrected for body surface area. The corresponding urinary clearance of PAH divided by the renal PAH-extraction ratio, was equated with renal plasma flow. The renal extraction ratio of PAH was determined immediately after completing the urinary clearances (during subsequent cardiac catherization) by sampling blood simultaneously from the femoral artery and the right renal vein. Renal vascular resistance was calculated by dividing the pressure drop from femoral artery to vena cava by renal blood flow, where the latter has been computed from renal plasma flow and the fractional hematocrit.

The concentrations of inulin and PAH were determined with an automated assay that has been described previously [2]. Plasma oncotic pressure was determined directly by membrane osmometry [3]. To permit accurate assay of albumin and immunoglobulin G (IgG) in unconcentrated urine, their respective concentrations were measured by a sensitive, enzyme-linked immunosorbent assay. Urine samples were placed in polystyrine wells coated with buffered antibodies to human albumin or IgG. Goat anti-human albumin or IgG conjugated with alkaline phosphatase was then added, followed by alkaline phosphatase substrate (Sigma Chemical Co. St. Louis, Missouri, USA). The color change was read from a standard curve on an optical densitometer at 490 nm (Micro-elisa Reader MR 600A, Dynatech Laboratories, Inc. Alexandria, Virginia, USA).

Plasma was thawed immediately before assay of plasma renin. Plasma renin activity was assayed as angiotensin I released from angiotensinogen during incubation of plasma at 37°C and pH 7.4. A renal renin secretion ratio (S_r) was calculated as

$$S_r = \frac{[V_r] - [A_r]}{[A_r]}$$

where $[V_r]$ is plasma renin activity of renal venous blood, and $[A_r]$ the corresponding value in femoral arterial blood. Additional aliquots of peripheral venous plasma were incubated with sheep angiotensinogen before and after dialysis to pH 3.3, to estimate active and total renin. Inactive renin activated at pH 3.3 was calculated as the difference between total and active renin [4].

Morphometric analysis

Twelve CsA-recipients agreed to undergo a renal biopsy on the day following the physiologic studies. Tissue was processed for light and fine structural study. For light microscopy the Zenker's fixed tissues were serially sectioned and stained with hematoxylin and eosin, PAS-alcian blue and Masson trichrome stains. Tubulointerstitial and vascular lesions were analyzed semiquantitatively, where 0 was no abnormality; 1+ to 4+ for vascular lesions, minimal to marked; and 1 to 4+ for tubulointerstitial lesions reflect the percentage of area of available tissue exhibiting tubular atrophy and interstitial fibrosis; 1 + was <25%, 2 + was 25 to 50%, 3 + was 50 to 75%, and 4 + was >75%. The outline of each glomerular tuft in three to five PAS-alcian blue stained sections per case was traced onto a digitizing tablet and its cross-sectional area determined by area perimeter analysis using the SMI micro-comp computerized system (Southern Micro Instruments Inc., Atlanta, Georgia, USA).

For electron microscopy tissue was fixed in gluteraldehyde, embedded in epon and stained with lead citrate. Survey sections were used to locate three glomeruli in each biopsy core that exhibited neither sclerosis nor ischemic collapse. Sixty to 70 nanometer sections of selected glomeruli were photographed at × 1000 and × 4000 and subjected to area perimeter analysis, as described in detail elsewhere [5]. Widening of the basement membrane was evaluated by determining the ratio of the total area-to-length of the glomerular basement membrane in each peripheral capillary loop. Changes in epithelial podocytes were assessed by counting the number of epithelial filtration slits per 1000 micrometers of capillary loop length. Finally, the fraction of total glomerular cross-sectional area occupied by mesangium was determined.

Renal tissue obtained from 10 living kidney donors was from Dr. Michael Steffes of the Department of Laboratory Medicine and Pathology at the University of Minnesota Hospital, Minneapolis, Minnesota, USA. All glomeruli in PAS-alcian blue stained sections of each biopsy were subjected to computerized area perimeter analysis at × 1000 magnification to provide

Table 1. Clinical features

	Azathioprine				Cyclosporine			
	Months post-transplant				Months post-transplant			
	1	6	12	24	1	6	12	24
Number surviving	94	63	59	50	95	84[a]	82[a]	73[a]
Immunosuppressive dosage *mg/kg/24 hr*	1.3 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	10.0 ± 0.4	7.4 ± 0.4	6.3 ± 0.3	5.3 ± 0.3
Prednisone dosage *mg/kg/24 hr*	1.01 ± 0.03	0.50 ± 0.03	0.39 ± 0.02	0.29 ± 0.02	0.47 ± 0.03[a]	0.23 ± 0.02[a]	0.18 ± 0.01[a]	0.16 ± 0.01[a]
Number on antihypertensives	0	1	3	3	53[a]	66[a]	66[a]	62[a]
Systolic BP	115 ± 1	121 ± 2	119 ± 2	125 ± 2	129 ± 2[a]	134 ± 2[a]	138 ± 2[a]	140 ± 2[a]
Diastolic BP	72 ± 1	83 ± 1	83 ± 2	84 ± 2	81 ± 1[a]	92 ± 1[a]	96 ± 1[a]	99 ± 2[a]
Serum creatinine *mg/dl*	1.0 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.0	1.3 ± 0.1[a]	1.9 ± 0.1[a]	2.1 ± 0.1[a]	2.0 ± 0.1[a]
Blood urea nitrogen *mg/dl*	27 ± 1	22 ± 1	20 ± 1	18 ± 1	39 ± 2	42 ± 1[a]	43 ± 2[a]	42 ± 2[a]

[a] Significantly different ($P < 0.05$) from corresponding value in azathioprine group

control values for glomerular tuft cross-sectional area. Two glomeruli from each biopsy were then prepared for electron microscopy as described above to provide control values for the ultrastructural morphometric quantities of interest.

Longitudinal examination

Fifteen CsA recipients examined initially 12 ($N = 9$) or 24 months ($N = 6$) after cardiac transplantation were restudied with identical physiologic techniques at the time of their routine 36 and 48 month, follow-up examinations. Sequential morphological observations were also made in six of the patients who had earlier undergone a renal biopsy. Four were members of the longitudinally studied group who exhibited increasing GFR at their 36 or 48 month sequential study. Renal tissue in the remaining two subjects was obtained at autopsy.

Statistical analysis

The significance of differences between the CsA and azathioprine subgroups, and of changes during the longitudinal study of CsA recipients was examined with an analysis of variance (ANOVA) using the CLINFO system.

Results

Clinical and Laboratory Findings

The 24 month outcome of cardiac transplantation in the 100 patient cohort treated with CsA is compared to that of the 100 patients treated earlier with azathioprine in Table 1. Patient survival was higher throughout in the CsA than in the azathioprine treated group, despite substantially lower maintenance doses of prednisone in the former. Whereas the need for antihypertensive agents was deemed rare in the azathioprine treated group, such therapy was administered within a month of transplantation to a majority, and by 12 or 24 months to more than 80% of CsA treated recipients. Antihypertensive therapy notwithstanding, average systolic blood pressure exceeded that in the azathioprine group by 13 to 19 mm Hg at each observation period; the corresponding excess of average diastolic blood pressure in the CsA group was by 9 to 15 mm Hg. The high prevalence of arterial hypertension in CsA treated recipients was accompanied by chronic azotemia. Whereas mean serum creatinine and blood urea nitrogen levels were within the normal range at all observation points in the azathioprine group, corresponding values were elevated at all time points in the CsA group.

Table 2. Initial physiologic findings

	Azathioprine recipients $N = 24$	Cyclosporine recipients $N = 37$	P value
Serum creatinine *mg/dl*	1.0 ± 0.04	1.8 ± 0.09	<0.001
Glomerular filtration rate *ml/min/1.73 m²*	94 ± 4	47 ± 3	<0.001
PAH extraction ratio	0.87 ± 0.02	0.75 ± 0.02	<0.001
Renal plasma flow *ml/min/1.73 m²*	450 ± 24	284 ± 19	<0.001
Filtration fraction	0.22 ± 0.01	0.16 ± 0.01	<0.001
Afferent oncotic pressure *mm Hg*	21.0 ± 0.5	21.6 ± 0.5	NS
Mean arterial pressure *mm Hg*	98 ± 2	115 ± 2	<0.001
Renal vascular resistance *units*	126 ± 7	272 ± 31	<0.001
Cardiac output *liter/min*	5.8 ± 0.3	5.7 ± 0.2	NS
Renal blood flow/cardiac output %	15.9 ± 1.2	8.8 ± 0.6	<0.001
Urinary albumin excretion *μg/min*	27 ± 14	132 ± 57	<0.01
Urinary IgG excretion *μg/min*	1.7 ± 0.6	7.8 ± 2.5	<0.02

Physiologic findings

On initial physiologic examination the 37 selected CsA recipients were receiving 6.1 ± 0.5 mg/kg per 24 hours and the trough level of immunoassayable CsA in serum was 164 ± 18 ng/ml. Their physiologic findings are compared to those of azathioprine treated controls in Table 2.The cardiac output in the two groups was not different, 5.7 ± 0.2 versus 5.8 ± 0.3 liter/min. Nevertheless, the GFR was reduced by half in the CsA recipient group, 47 ± 3 versus 94 ± 4 ml/min per 1.73 m² ($P < 0.001$). Although renal plasma flow was reduced in parallel, glomerular underperfusion alone cannot account entirely for the lowered GFR. This is indicated by a significantly depressed filtration fraction, 0.16 ± 0.01 versus 0.22 ± 0.01 ($P < 0.001$).

Table 3. Renin angiotensin system

	Azathioprine recipients	Cyclosporine recipients[a]	*P* value
Plasma renin activity[b] *ng/ml/hr*	3.6 ± 1.3	1.1 ± 0.3	0.05
Renin secretion ratio	0.31 ± 0.10	0.44 ± 0.13	NS
Total renin *ng/ml/hr*	182 ± 43	230 ± 44	0.05
Active renin[b] *ng/ml/hr*	14.6 ± 4.6	5.9 ± 0.7	<0.01
Inactive renin[b] *ng/ml/hr*	167 ± 39	224 ± 44	<0.02
Inactive/active renin	13.3 ± 1.5	27.8 ± 3.5	<0.01

[a] Eight cyclosporine recipients receiving antihypertensive treatment with captopril have been excluded from the analysis.

[b] Normal supine values in aged-matched healthy individuals on ad libitum diet are: plasma renin activity 0.1–2.2, active renin 0.4–14.0, and inactive renin 11–49 ng/ml/hr, respectively.

Additional evidence of glomerular injury in CsA recipients was a modest elevation of urinary albumin excretion rate above the azathioprine control level, 132 ± 57 versus 27 ± 14 μg/min ($P < 0.01$). A corresponding increase in urinary IgG excretion rate was also observed, 7.8 ± 2.5 versus 1.7 ± 0.6 μg/min ($P < 0.02$). Afferent oncotic pressure (which is taken to be the same as in peripheral venous blood) was similar in the two groups, but mean arterial pressure in CsA recipients was elevated by 17 mm Hg above that in those receiving azathioprine ($P < 0.001$). Renal vascular resistance was twofold elevated in CsA recipients, 272 ± 31 versus 126 ± 7 (mm Hg · min) per liter in azathioprine recipients ($P < 0.001$). That increased resistance in the renal vascular bed associated with CsA therapy was relatively selective is indicated by the corresponding percentage of cardiac output perfusing the kidneys in the two groups, 8.8 ± 0.6 versus 15.9 ± 1.2%, respectively.

In keeping with earlier reports, plasma renin activity and inactive renin remained strikingly elevated (4.2 ± 1.2 and 403 ± 102 ng/ml per hr) in eight members of the CsA group who had been receiving therapy with captopril [6, 7], even though antihypertensive therapy had been withdrawn four days previously. When these eight subjects were excluded, plasma renin activity was lower in CsA than Aza recipients, 1.1 ± 0.3 versus 3.6 ± 1.3 ng/ml per hr ($P = 0.05$, Table 3). In contrast the renal renin secretion ratio was similar in the two groups, 0.44 ± 0.13 versus 0.31 ± 0.10, respectively. When taken together with the 37% reduction of renal plasma flow however, the latter finding suggests that the renal production rate of active renin was, if anything, lower in CsA than azathioprine recipients. Total plasma renin concentration was high in both groups, due largely to a striking three- to fivefold increase in inactive renin above normal levels (Table 3). CsA recipients differed from azathioprine controls however, in that active renin was significantly lower, while the opposite was true of inactive renin. These differences are reflected by a twofold elevation ($P < 0.01$) of the inactive:active plasma renin ratio in CsA versus azathioprine recipients (27.8 ± 3.5 and 13.3 ± 1.5, respectively), and suggest that chronic CsA therapy is associated with a partial block in the intrarenal conversion of inactive prorenin to active renin.

Morphology

Light microscopy. The light microscopic findings in the 12 initial biopsy samples are included in Table 4 (cases 1 to 12). Glomeruli in 9 of the 12 instances exhibited global or segmental sclerosis involving from 2 to 34% of the glomeruli (Fig. 1A). In keeping with the increased synthesis of inactive prorenin, striking juxtaglomerular apparatus hyperplasia was evident in eight patients. Collapsed glomeruli which appeared smaller than normal were prominent in 9 of the 12 biopsies, comprising 6 to 56% of all glomeruli in these biopsy cores (Fig. 1B). Six hundred sixty-five glomerular cross sections were available for morphometric analysis in the 12 biopsy cores of CsA recipients, while the corresponding number of glomerular cross sections in normal control tissue was 731. The distribution of glomerular tuft cross-sectional area in CsA-treated patients is compared to that of normal controls in Figure 2. Tuft area was distributed around a mode of 12000 μ^2 in the control group. However, the rather bell-shaped distribution apparent in controls was lost in CsA recipients. The percentage of glomeruli with cross sectional area below the mode was increased in CsA recipients (53 vs. 45% in controls) reflecting presumably collapsed and sclerosed glomeruli. A corresponding increase in glomerular tufts of cross sectional area above 20000 μ^2 (11 vs. 4%) suggests that spared, remnant glomeruli had become enlarged (Fig. 1).

There were subtle to conspicuous arteriolar abnormalities in all but one case (Fig. 3). These consisted of subintimal and medial hyalin deposits indistinguishable from the hyalin deposits seen in the afferent and efferent arterioles of diabetic, hypertensive and nephrotic patients (Fig. 3A). There were also frequent examples of the fibrointimal thickening and elastic membrane replication encountered as age related and hypertensive phenomena. The former occurred in eleven and the latter in seven of the cases. A more distinctive vascular lesion, the so-called CsA-associated arteriolopathy, involved the afferent arterioles [8]. This appeared as an extensive eosinophilic, lumpy and smudgy deposit which replaced the pericytes of the arteriole. When minimal, its appearance was similar to the

Table 4. Histopathologic features of cyclosporine nephrotoxicity

Case	Source	Months on CsA	GFR	GS%	IG%	CTI	HYA	CsA-AA	JGA
1a	BX1	24	39	0	0	1+	1+	0	3+
1b	BX2	48	53	10	24	2+	1+	2+	0
2a	BX1	24	33	0	18	2+	0	0	1+
2b	BX2	48	40	15	33	2+	2+	3+	1+
3a	BX1	12	32	5	0	2+	0	0	1+
3b	BX2	24	34	15	9	3+	1+	0	0
4a	BX1	24	52	9	36	1+	4+	3+	0
4b	BX2	36	68	18	46	2+	4+	3+	0
5a	BX1	12	52	5	14	2+	1+	1+	1+
5b	AUT	19	?	11	15	1+	0	1+	2+
6a	BX1	24	16	18	18	2+	1+	1+	0
6b	AUT	28	0	23	30	2+	2+	2+	3+
7	BX1	12	37	34	56	3+	1+	2+	0
8	BX1	24	28	25	17	1+	1+	1+	0
9	BX1	12	33	3	0	1+	1+	2+	1+
10	BX1	48	33	2	38	3+	2+	2+	1+
11	BX1	36	45	8	6	2+	2+	2+	1+
12	BX1	24	50	0	0	1+	1+	1+	2+
13	BX1	60	15	76	9	3+	3+	3+	0
14	AUT	12	0	12	80	2+	1+	1+	1+
15	BX1	9	17	20	25	3+	1+	2+	1+

Abbreviations are: BX, biopsy; AUT, autopsy; GFR, glomerular filtration rate; GS, glomerulosclerosis; IG, ischemic glomerular collapse; CTI, tubular atrophy, interstitial fibrosis; HYA, arteriolar hyalinosis; CsA-AA, cyclosporine-associated arteriolopathy; JGA, juxtaglomerular apparatus hyperplasia. In cases 1-6, a is initial and b is follow-up examination.

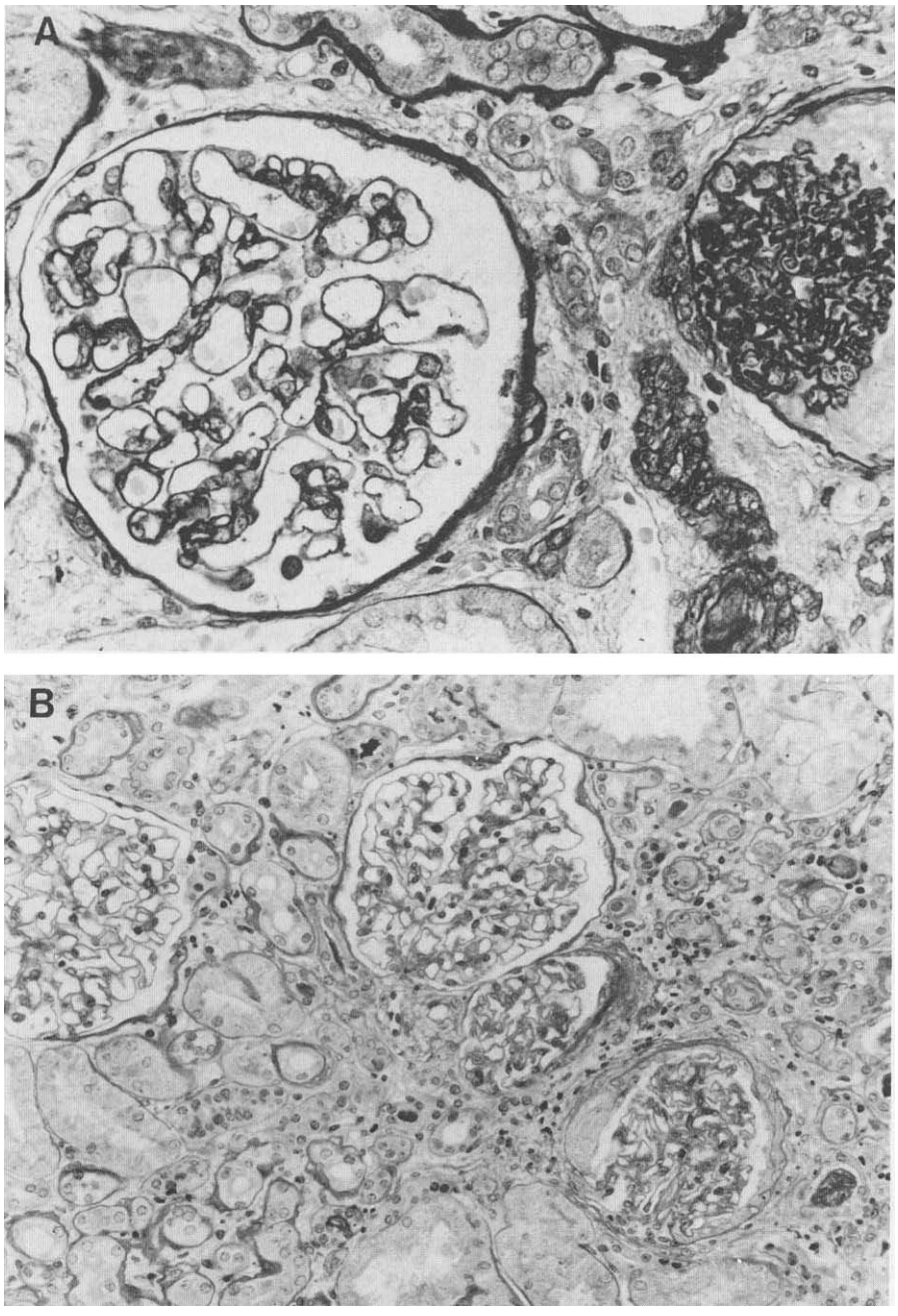

Fig. 1. A. *Global glomerular sclerosis (PAS-alcian blue × 200).* **B.** Small glomeruli that had undergone ischemic collapse (PAS-alcian blue × 400) were prevalent in both first and second biopsies. Some glomeruli that have escaped these changes appear to have undergone hypertrophy.

hyalin deposit commonly associated with diabetes mellitus, but when conspicuous, its appearance was more distinctive (Fig. 3B). In most cases individual pericytes showed considerable anisonucleosis (Figs. 1A, 3A), and in some the media was nearly devoid of pericytes even when smudgy deposits were inconspicuous. An additional feature was the finding of karyorrhectic debris in the media in three cases. Fibrinoid necrosis and fibrin thrombosis of vessels were not observed in any of the cases.

Associated with the glomerular sclerosis, ischemic glomerular tufts and vascular lesions were variable tubular atrophy, interstitial fibrosis and chronic inflammation (Figs. 1 and 3). In some cases the changes were nearly diffuse, but most exhibited narrow stripes of atrophy and fibrosis, apparently corresponding to areas of cortex associated with arteriolar lesions.

Electron microscopy. Three glomeruli exhibiting no evidence of either sclerosis or collapse at × 1000 magnification were selected from each biopsy core. Electron photomicrographs were then subjected to area perimeter analysis. The ratio of basement membrane area to total length of all glomerular capillary loops in the tuft was slightly but not significantly increased above control values, 0.54 ± 0.02 versus 0.49 ± 0.01 (P = NS) suggesting only mild thickening of the glomerular basement membrane. Similarly a patchy broadening of epithelial foot processes lowered slightly the frequency of filtration slits to 1.0 ± 0.04 per 1000 micrometers of capillary loop length

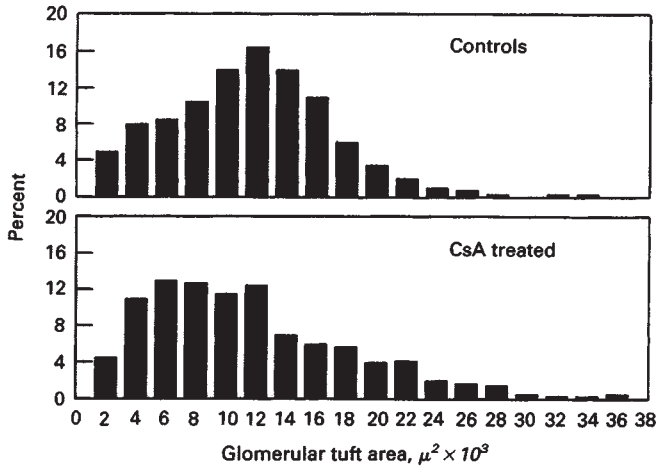

Fig. 2. *Distribution of surface area of 731 glomerular cross sections in biopsy cores of 10 controls (upper histogram) is compared to that of 665 glomerular cross sections in first biopsy cores of 12 recipients who had received CsA for between 12 and 24 months (lower histogram).* Note glomerular cross-sectional area is shifted towards both smaller and larger size in CsA treated recipients.

versus 1.2 ± 0.07 in controls (P = NS). A diffuse expansion of the mesangium reflecting matrix more than cellular components, constituted the most striking ultrastructural alteration. Fractional mesangial area varied from 0.16 to 0.20 in the 10 control glomeruli, but exceeded 0.20 in glomeruli of all but two CsA recipients (Fig. 4, range 0.14 to 0.39). On average, fractional mesangial area was increased significantly, 0.23 ± 0.02 versus 0.17 ± 0.007 ($P < 0.01$). Because total cross-sectional area of selected glomeruli was larger in CsA recipients than controls, we infer that chronic administration of CsA is characterized by a generalized accumulation of mesangial matrix in glomeruli that have neither collapsed nor undergone global sclerosis.

Longitudinal findings

Fifteen patients studied initially at 12 or 24 months were reexamined at 36 and again at 48 months post-transplant. Their physiologic findings are summarized in Table 5. CsA was withdrawn because of severe renal insufficiency and replaced by azathioprine in one instance after 18 months and in an additional three cases after 36 months. In the remaining patients CsA dose was lowered progressively from an initial dose of 6.8 ± 0.6 to 4.5 ± 0.5 and 3.1 ± 0.6 mg/kg per 24 hours, by 36 and 48 months, respectively. The corresponding trough levels of CsA in serum declined in parallel, from 175 ± 23 to 118 ± 21 and 66 ± 16 ng/ml, respectively. Judged by a trend towards increasing cardiac output and declining left atrial pressure, cardiac performance improved in the interim. Despite withdrawal or reduction of CsA and improving cardiac performance, renal plasma flow tended to be lower and renal vascular resistance higher at the two follow-up examinations. GFR remained constant at 56 ± 4, 54 ± 7 and 55 ± 5 ml/min per 1.73 m^2, perhaps facilitated by a significant decline in serum protein concentration with a corresponding decline in average plasma oncotic pressure by 1.3 to 1.5 mm Hg at follow-up examination. Arterial pressure remained elevated throughout, 118 ± 4 initially and 115 ± 4 and 116 ± 4 mm Hg at 36 and 48 months, respectively.

Inspection of the individual determinations of GFR in Figure 5 reveals quite large fluctuations in this quantity over a 48 month period, suggesting a functional component to the hypofiltration. However, only occasional examples of GFR entering the control (azathioprine recipient) range were encountered. Moreover, GFR failed to return to the control range in the four patients in whom CsA was withdrawn completely (dashed lines, Fig. 5). In fact, GFR declined below earlier values in two of these latter subjects despite withdrawal of CsA, indicating fixed and progressive injury to glomeruli. In keeping with progressive glomerular injury, increasing proteinuria of variable magnitude became manifest (Fig. 6). On average, the excretion rate of albumin increased significantly from an initial value of 162 ± 70 to 396 ± 164 at 36 months ($P < 0.05$) and 546 ± 300 μg/min at 48 months ($P < 0.01$). Corresponding increases were observed also in the urinary excretion rate of IgG, from 6 ± 2 to 21 ± 7 ($P < 0.01$) and 53 ± 43 μg/min ($P < 0.05$), suggesting increasing impairment of glomerular barrier size-selectivity with the passage of time [5].

A subsequent tissue sample was obtained 6 to 24 months after the initial biopsy in cases 1 through 6 (Table 4). There was evidence of progressive renal damage without exception. An increase was observed in the percentage of glomeruli exhibiting either sclerosis (6.2 ± 6.7 to 15.3 ± 4.8%; $P < 0.001$) or ischemic collapse of the tuft (14.7 ± 12.5 to 24.2 ± 13.5; $P < 0.02$). Atrophic tubular changes and interstitial fibrosis also increased (1.5 ± 0.5 to 2.0 ± 0.6; P = NS). CsA-associated arteriolopathy became manifest or more conspicuous between the two examinations in four of six instances (0.5 ± 0.5 to 1.8 ± 1.2; $P < 0.05$); there was a parallel increase in arteriolar hyalinosis. Particularly noteworthy is that the four patients subjected to repeat biopsy (cases 1 to 4, Table 4) were selected because the GFR was actually slightly higher at the time of the second biopsy. Notwithstanding the modestly improved level of GFR, the second biopsy revealed a substantial increase in the percentage of glomeruli that had undergone either sclerosis or ischemic collapse. Thus serial measurement of the GFR does not appear to reflect reliably a progressive obliteration and sclerosis of the cortical microvascular bed in the kidneys of patients receiving CsA.

Development of end-stage renal disease

Three of the 37 member CsA subgroup subjected to physiological study have since developed end-stage renal failure. One (case #6, Table 4) examined on a single occasion after 24 months of CsA treatment, required dialysis after 26 months. The second, (case #8, Table 4 and depicted by the lowest recorded GFR at 36 and 48 months in Fig. 5) received a renal transplant after 50 months. The third, (case #13, Table 4) was found to have the most extensive glomerulosclerosis and arteriolopathy in our entire series. His biopsy was performed 79 months following transplantation, the first 60 of which were associated with continuous CsA therapy. Although azathioprine was substituted for CsA after 60 months, his renal function continues to deteriorate and he is currently being prepared for a renal transplantation.

Of the remaining 63 cardiac allograft recipients treated with CsA and not subjected to physiological study during the course

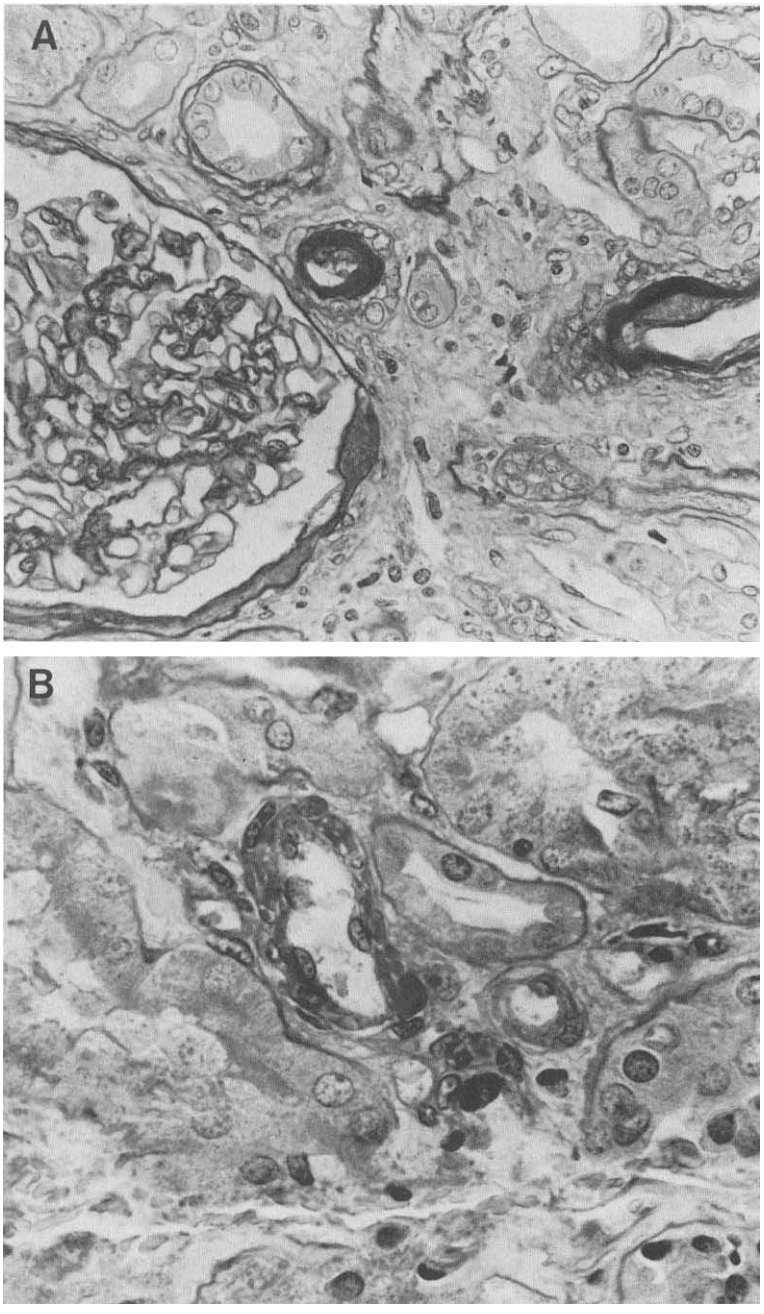

Fig. 3. A. *The appearance of arteriolar hyalinosis is due to deposits of PAS positive material in a subintimal location (PAS-alcian blue × 400).* **B** An example of CsA-arteriolopathy is demonstrated, in which the PAS positive material is seen replacing pericytes (PAS-alcian blue × 640).

of this study, 33 are known to have survived more than two years at the time of writing. Two of these have documented evidence of having developed end-stage renal disease requiring dialysis therapy. One (case #14, Table 4) had histopathologic features at autopsy similar to those observed in biopsy material from our study population. The second (case #15, Table 4) was biopsied at another center because of declining renal function after nine months of CsA therapy. Review of the tissue revealed platelet-fibrin thrombi in glomerular capillaries and arterioles consistent with the hemolytic-uremic syndrome. Although this process appears to be idiosyncratic, it is well recognized as an adverse effect of CsA on the kidney [9]. Thus, there was a small but definite risk of early progression to end-stage renal disease within four years among cardiac transplant recipients treated chronically with CsA. In contrast, chronic renal failure progressing to end-stage renal disease has never been observed among 148 cardiac transplant recipients treated only with azathrioprine and prednisone at this institution between 1969 and 1980.

Discussion

A large body of evidence indicates that the daily use of CsA for 12 months or longer is associated with a chronic renal injury. Most experience with the protracted use of CsA has been in

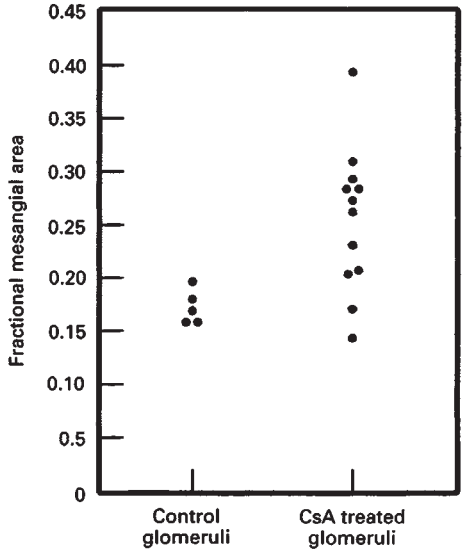

Fig. 4. *Fraction of glomerular tuft cross-sectional area occupied by mesangium in glomeruli of control (left) and CsA-treated recipients* (right).

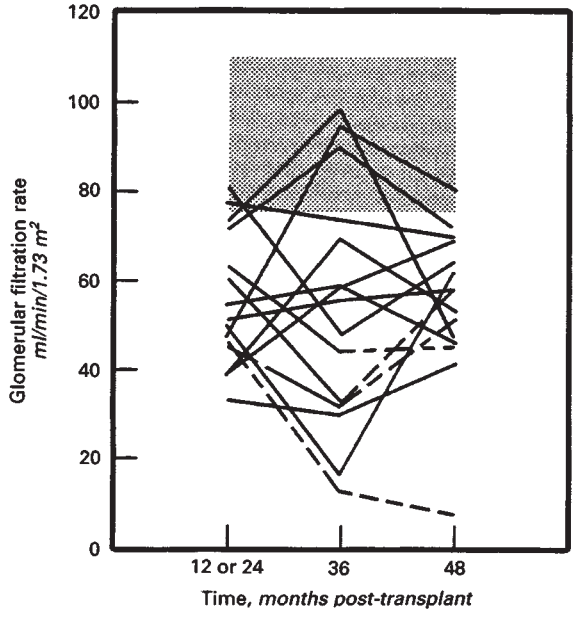

Fig. 5. *Serial determinations of GFR in 15 CsA-treated cardiac transplant recipients.* The shaded area is the range exhibited by azathioprine treated controls. Dashed lines reflect intervals and examinations following withdrawal of CsA therapy in four cases.

Table 5. Longitudinal physiologic findings

	Initial *12 or 24 months*	36 Months	48 Months
CsA dose *mg/kg/24 hr*	6.8 ± 0.6	4.5 ± 0.5[c]	3.1 ± 0.6[d]
CsA trough *ng/ml*	175 ± 23	118 ± 21[b]	66 ± 16[b]
Cardiac output *liter/min*	5.8 ± 0.2	6.1 ± 0.4	6.3 ± 0.3[a]
Left atrial pressure *mm Hg*	12 ± 1	10 ± 1	8 ± 1[a]
GFR *ml/min/1.73 m²*	56 ± 4	54 ± 7	55 ± 5
Renal plasma flow *ml/min/1.73 m²*	312 ± 13	272 ± 30	293 ± 43
Renal vascular resistance *(mmHg · min)/ml*	212 ± 8	265 ± 31[a]	249 ± 40
Mean arterial pressure *mm Hg*	118 ± 4	115 ± 4	116 ± 4
Afferent oncotic pressure *mm Hg*	22.6 ± 0.5	21.1 ± 0.6[b]	21.3 ± 0.6
Serum protein concentration *g/dl*	6.5 ± 0.1	6.0 ± 0.1[d]	6.0 ± 0.1[b]
Urinary albumin excretion	162 ± 70	396 ± 164[b]	546 ± 300[c]
Urinary IgG excretion	6 ± 2	21 ± 7[c]	53 ± 43[b]

[a] $0.05 > P < 0.1$ vs. initial study
[b] $P < 0.05$ vs. initial study
[c] $P < 0.01$ vs. initial study
[d] $P < 0.001$ vs. initial study

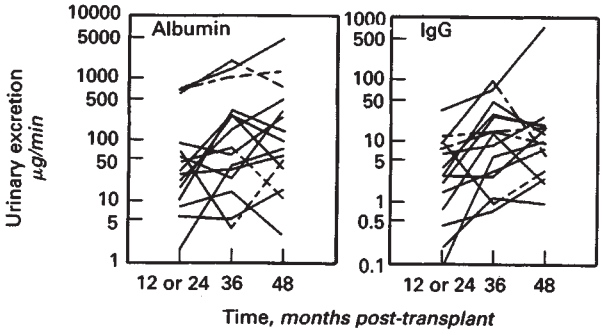

Fig. 6. *Serial urinary excretion rate of albumin* **A** *and immunoglobulin G* **B** *in 15 CsA-treated recipients.* Dashed lines reflect intervals and examinations following withdrawal of CsA therapy in four cases. Note differences in the logarithmic scales on each ordinate.

renal allograft recipients, in whom the differentiation of CsA nephropathy from chronic allograft rejection has been difficult [8, 10]. However, a chronic renal injury with characteristics similar to those described in the present study has been reported also in liver allograft recipients [11] and in patients with uveitis [12], who like cardiac allograft recipients, have healthy native kidneys. The absence of discernable chronic renal damage in the foregoing patient categories when treated by alternative immunosuppressive regimens, suggests that CsA therapy itself is responsible for the renal injury.

The dosage of CsA employed by us perioperatively, and the maintenance doses used in the first two years of our experience with this agent, are today regarded by many as excessive. Of note however, the use of similar dosing schedules in two large multicenter trials in renal transplant recipients was associated also with persistent elevation of the mean serum creatinine level [13, 14]. On the basis of a rather stable average level of azotemia in these, and other large groups of renal transplant recipients beyond two years of therapy, it has been argued that a progression of chronic nephropathy does not occur in patients receiving CsA in doses comparable to those used by us [13–16]. Our finding that average GFR remains stable, albeit severely depressed, for up to 48 months of CsA treatment, explains the above-cited constancy of the level of azotemia. The demonstration of a chronically stable level of glomerular hypofiltration does not appear to rule out progressive renal injury, however.

Our histopathologic observations suggest that the cortical microvasculature undergoes progressive damage and obliteration with continuous administration of CsA beyond 12 months. Arteriolar abnormalities including pericyte hypertrophy, necrosis and replacement of the wall with proteinaceous deposits are most likely the result of CsA-induced vasospasm. Irreversible ischemic damage appears to occur following replacement of the media by fibrous tissue, with secondary damage to glomeruli and tubular loss [8]. In keeping with this possibility is our demonstration of a trend towards higher renal vascular resistance between 24 and 36 months of therapy (Table 5). RVR failed to increase further between 36 and 48 months, during which interval CsA was discontinued in three patients. This finding suggests that elevated arteriolar resistance is in part attributable to functional vasospasm as well as to luminal obliteration.

Withdrawal of CsA after a few months of therapy has been associated with reversal of azotemia [17, 18]. Even when serum creatinine levels have remained unchanged, withdrawal of CsA after less than 12 months of therapy has been shown to be followed by an increase in renal plasma flow [19]. The disparity between these short term findings and our observation of a late increase in renal vascular resistance can be reconciled if renal vasospasm early in the course of CsA therapy gives way, with the passage of time, to a structural obliteration of the renal cortical vascular bed.

The relative constancy of GFR despite an apparently progressive obliteration of cortical microvessels is intriguing. It suggests that the single nephron GFR of remnant glomeruli that remain perfused is adaptively increased, thereby compensating for the declining number of functional nephrons. In keeping with this hypothesis, some preserved glomeruli in our biopsy material were larger than normal (Fig. 2). Like remnant glomeruli of the rat following extensive ablation of renal mass [20], they also exhibited a generalized expansion of mesangial matrix (Fig. 4), increasing leakiness of their capillary walls to large plasma proteins (Fig. 6) and ultimately, increasing sclerosis (Table 4). Whether arterial hypertension is also an expression of a declining number of nephrons, or a consequence of a direct action of CsA on the systemic vasculature to elevate peripheral resistance, cannot be determined. Transmission of elevated arterial pressure to glomerular capillaries has been a consistent feature of remnant glomeruli however, and has been incriminated in driving the sclerosing process in surviving glomeruli [21–23].

Short term studies in the rat suggest that CsA can activate the renin-angiotensin system acutely, and that this vasopressor system may play an initiating role in the genesis of CsA associated nephropathy [24, 25]. Our finding of extreme elevation of plasma inactive renin (which closely resembles prorenin) and of total plasma renin, together with prominent hyperplasia of juxtaglomerular apparati, is consistent also with a more chronic stimulation of the renin-angiotensin system (Table 3). In keeping with other reports however, we do not find hypertension late in the course of the renal injury to be associated with enhanced plasma renin activity [26–28]. The combination of increased synthesis of prorenin with a limited release of active renin could be explained by a partial block in the intrarenal conversion of the prohormone to active renin. Of interest, the combination of high plasma inactive renin with normal or low active renin is also found in the sclerosing glomerulopathy of diabetes mellitus [4, 29], a condition characterized by a diffuse expansion of mesangial matrix and arteriolar hyalinosis like that observed with chronic CsA therapy.

An especially discouraging feature of our longitudinal study is that chronic renal injury, once established, cannot be reversed by either substantial reductions in the dosage of CsA or even its complete withdrawal. That the chronic nephropathy may in fact be progressive is suggested by our serial histopathological observations and by the development of end-stage renal disease in 5 of 73 CsA-treated recipients, who have survived cardiac transplantation for two years or longer. Since it takes typically more than a decade for a variety of chronic renal injuries to destroy the kidney, it could be that these isolated examples represent merely the vanguard of patients with CsA-associated chronic nephropathy who are destined to progress irrevocably, but more slowly, to end-stage renal disease. In light of this potential outcome, it is germane to question whether the rapidly proliferating use of CsA outside of the field of organ transplantation is justified. The dilemma posed by such expanded use of CsA is well exemplified by therapeutic trials in two immunologic disorders that have the potential to be complicated by sclerosing glomerulopathy, namely type I diabetic insulitis and minimal change nephropathy [30–32]. Glomerulosclerosis (initially focal) leading ultimately to end-stage renal disease is likely to occur in only a very small minority of patients with minimal change nephropathy [33], and only after a decade or more in fewer than 50% of type I diabetics [34]. Because these disorders recur promptly when CsA is withdrawn, protracted or even permanent administration of this agent is likely to be required [32, 35]. Although it is possible that therapeutic efficacy can be achieved at lower doses than those used in the first part of the present study, it remains to be demonstrated convincingly that lower dosage schedules are free of long-term, nephrotoxic effects [36]. Based on our present knowledge, it is by no means certain that CsA induced injury will not lead to end-stage renal disease more rapidly and commonly than the aforementioned diseases, a therapeutic outcome that would clearly be self-defeating.

The uncertainty surrounding the ultimate outcome on the kidney of long-term CsA therapy is heightened by the lack of a non-invasive procedure with which to monitor possible progression of the renal injury. Our experience indicates that in most instances accurate, repeated determinations of the GFR may not rule out a progressing injury. Although more discriminating, even invasive procedures such as renal biopsy and the determination of renal vascular resistance are problematic. The former is subject to error because random samples of the renal cortex may not reflect accurately the injurious process, which has a patchy distribution. The need to catheterize the renal vein so as to estimate PAH extraction reliably precludes the use of PAH clearance alone to determine renal vascular resistance. PAH extraction by chronically damaged kidneys of the present study was impaired and unpredictable, spanning a range of 0.48 to 0.96 and averaging 0.78.

We recommend that researchers intensify their efforts to better characterize the course and dose dependency of CsA-associated chronic nephropathy, to seek clinically applicable methods to detect unusually susceptible subjects at especially

high risk of a lethal renal injury, and to find ways to safely convert recipients of stable allografts from CsA to other forms of immunosuppression within a year of transplantation. In the meantime, we propose that the use of CsA be limited to recipients of allografts and those with immunologically-mediated diseases that are sufficiently severe to threaten life in the near term.

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