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Chronic rejection of rat renal allograft

I. Histological differentiation between chronic rejection and cyclosporin nephrotoxicity

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Abstract. Chronic allograft rejection is both a clinical and a histopathological diagnosis. Until recently, the histological definition of chronic renal allograft rejection was based on clinical diagnostic biopsies, where the evidence was partially obscured by recurrence of the original renal disease, and/or by administration of immunosuppressive drugs. In this communication, we present an experimental rat model for chronic renal allograft rejection, devoid of recurrence of the original disease. By comparing allografts to similarly immunosuppressed syngeneic transplants, we define which histological features should be attributed to chronic rejection and which to cyclosporin nephrotoxicity. Rat renal transplants were performed from DA (Ag-B4, $RT1^{av1}$) to WF strain (Ag-B2, $RT1^{u}$) or, for control, to DA strain, and immunosuppressed for 2 or 3 weeks with cyclosporin using a variety of different dosages. The animals were monitored weekly for serum creatinine levels and for blood cyclosporin concentrations, and core needle biopsies were performed on the grafts at regular intervals. At 3 months post-transplantation the animals were sacrificed and a complete histopathological evaluation was performed. Thirty-one histological variables were scored blindly by two investigators and separately for the graft interstitium, glomeruli, tubuli, and the graft vasculature. The following histological alterations were significantly more prominent in allografts than in similarly immunosuppressed syngeneic transplants: the intensity of interstitial inflammation, particularly the degree of pyroninophilia within the inflammatory cell population; the extent of glomerular mesangial matrix increase, basement membrane thickening, and glomerular sclerosis; the increase in the vascular intimal thickness affecting in particular the first and second order branches of the renal artery; and the obliteration of the graft vasculature. These alterations were considered as being primarily due to chronic rejection. In contrast, the extent of interstitial fibrosis and the extent of tubular changes, including tubular epithelial vacuolation, epithelial atrophy, and tubular basement membrane changes, were not significantly different in the allografts as compared to the syngeneic controls. These alterations were attributed primarily to cyclosporin nephrotoxicity. Serial monitoring of the grafts by needle biopsies clarified the sequence of events in the development of the chronic alterations in the transplant. The first event, as expected, was tubulointerstitial pyroninophilic inflammation, resembling that of acute episodes of rejection. This was significantly stronger and appeared earlier in allografts immunosuppressed for 2 rather than for 3 weeks. Vascular alterations developed next. The last to develop were the glomerular lesions.

Key words: Chronic rejection, rat model – Rejection, chronic, rat model – Cyclosporin toxicity, chronic rejection, in the rat

Short-term results of organ transplantation have dramatically improved during the past years. Most centers report approximately 75%–85% 1-year survival rates for most first cadaveric allografts, including the kidney [4]. A different problem has now evolved, namely, that of chronic rejection, affecting transplants during the later post-transplantation period.

Chronic rejection is both a clinical and a histopathological diagnosis. This form of rejection causes a progressive decline in graft function and is associated with a slowly increasing plasma creatinine concentration against time.

The main histological features attributed to chronic kidney allograft rejection are: ongoing perivascular and interstitial inflammation, fibrosis, glomerular sclerosis, vascular intimal hyperplasia, and tubular atrophy [5, 22]. These features overlap with the histological features of acute vascular rejection, cellular tubulointerstital rejection, and/or various types of glomerulopathy. The main differential diagnostic problem with renal transplants is finding a way to exclude recurrence of original disease and late effects of cyclosporin nephrotoxicity [17, 18].

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Table 1. Parameters evaluated in the histological specimens of renal grafts. All specimens were scored blindly from 0 to 3, with 0 indicating no pathological alterations and 3 indicating extreme changes

Glomeruli ($n = 10$)[a]	Tubuli ($n = 9$)[b]
Number of glomeruli	Epithelial swelling
Mesangial cell proliferation	Epithelial vaculation
Mesangial matrix increase	Epithelial desquamation
Capillary basement membrane thickening	Epithelial atrophy
Capillary basement membrane duplication	Epithelial necrosis
Capillary thrombosis	Casts
Bowman capsular thickening	Inflammation
Glomerular inflammation	Dilatation
Glomerular sclerosis	Basement membrane thickening
Glomerular necrosis	
Interstitium ($n = 6$)	**Vessels ($n = 6$)[c]**
Inflammation	Endothelial swelling
Pyroninophilic cells (%)	Endothelial proliferation
Edema	Intimal proliferation
Hemorrhage	Inflammation
Fibrin deposits	Sclerosis
Fibrosis	Obliteration

[a] Number of parameters evaluated
[b] Separately for proximal and distal tubules
[c] Separately for arteries, veins, and arterioles

Although chronic rejection is a well-established clinical entity in renal transplants and primarily responsible for late graft losses, it has been largely neglected in the medical literature. Apart from the information deriving from human renal transplant biopsies, very little experimental evidence exists on the pathogenesis of this disorder [21, 26]. The cellular and molecular level cascades leading to chronic rejection are virtually unknown, and no effective prophylaxis or treatment of chronic rejection has been designed.

In this communication we present an experimental model for chronic renal allograft rejection, employing inbred rat strains and a short period of initial immunosuppression with cyclosporin. Based on repeated biopsies of the rat renal allografts, we also describe the sequence of events in the generation of the histopathological lesions characteristic of chronic rejection.

Materials and methods

Animals

Male inbred WF (Ag-B2, RT1^u) and DA (Ag-B4, RT1^{av1}) rats were purchased from the Zentralinstitut für Versuchstierzucht (Hannover, FRG). The rats weighed 200–250 g and were 2–3 months of age.

Transplantations

Transplantations were performed from DA-to-WF strain (allogeneic) or from DA-to-DA strain (syngeneic), using the modified technique described by Fisher and Lee [8]. The animals were anesthetized with an intraperitoneal injection of chloral hydrate solution (250 mg/kg). The donor right kidney, ureter, renal artery and vein were prepared free from the surrounding tissues. The suprarenal artery and superior mesenteric artery were ligated and severed. The aorta was clamped on the level of the celiac artery. The kidney was then flushed with 5 ml of 0.9% saline containing 10 IU of heparin (Heparin, Medica, Helsinki, Finland) per milliliter until the kidney was macroscopically bloodless. The kidney and 1 cm of ureter were removed en bloc, including the renal artery with a 3-mm aortic cuff and the renal vein with a 3-mm vena cava cuff.

The kidney was placed into an isotonic saline bath at 4 °C. The recipient right kidney was removed, leading the ureter as long as possible. The donor kidney was transplanted to the recipient's abdominal aorta and inferior vena cava below the left renal artery by end-to-side anastomoses with the use of 9-0 nonabsorbable mono-

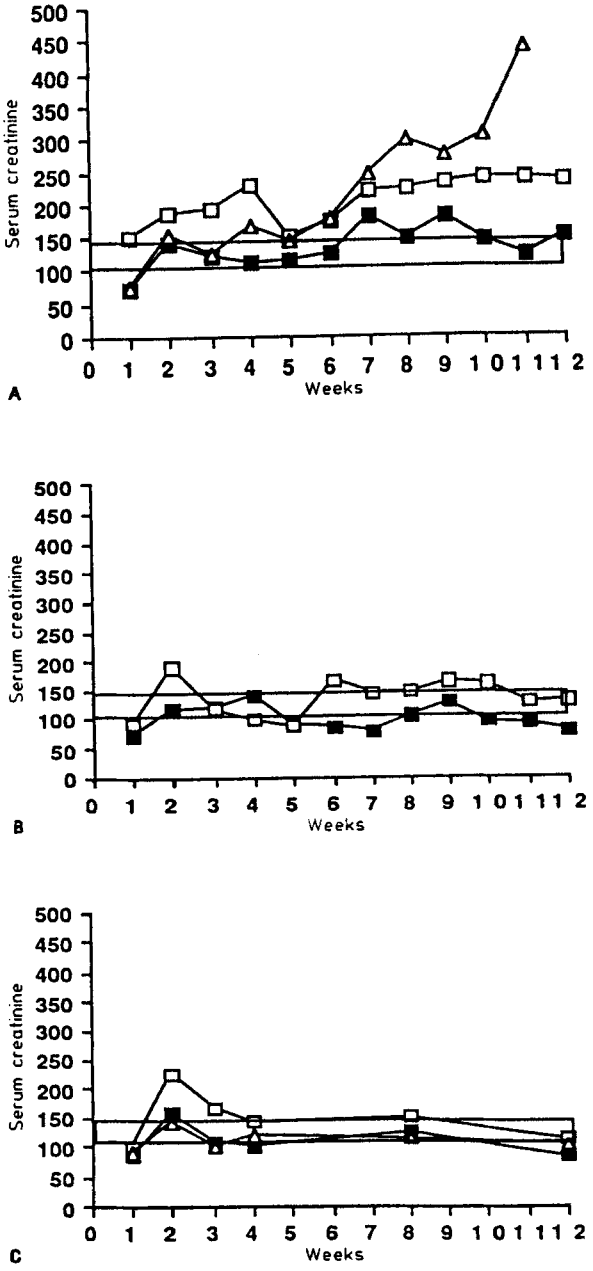


Fig. 1A–C. Mean serum creatinine (μmol/l) in: **A** rat renal allograft recipients receiving 5, 10, or 15 mg/kg per day of CyA for 2 weeks; **B** in rat renal allograft recipients receiving 5 or 10 mg/kg per day of CyA for 3 weeks; and **C** in syngeneic graft recipients receiving 5, 10, or 15 mg/kg per day of CyA for 3 weeks. All points represent the mean values of five separate transplants. ■ 5 mg/kg; □ 10 mg/kg; △ 15 mg/kg

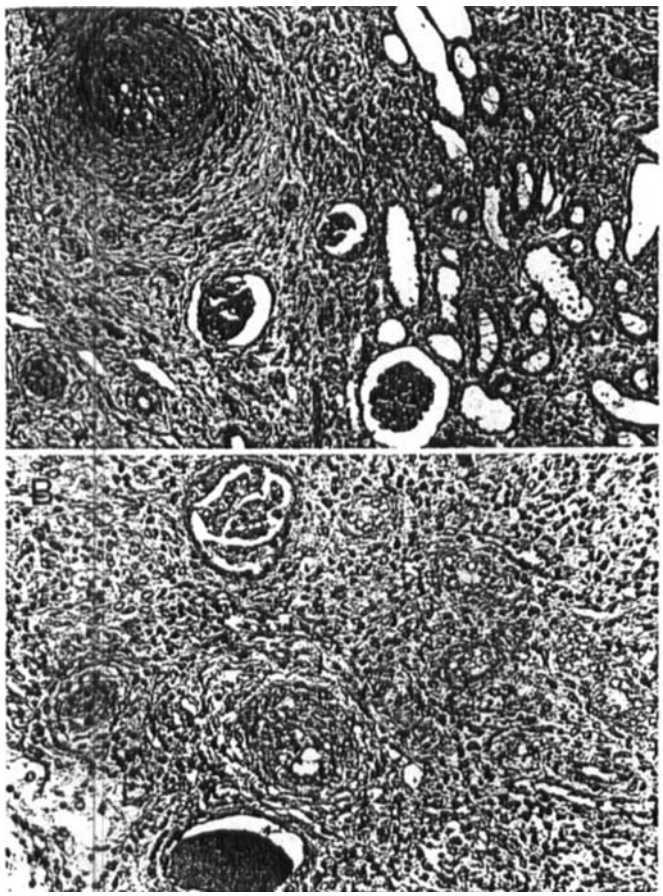


Fig. 2. A, B. General view of a long-surviving DA-to-WF rat renal allograft: **A** outer cortex; **B** inner cortex (periodic acid-Schiff × 50)

filament nylon suture and an atraumatic needle. Ureter anastomosis was performed end-to-end close to the renal pelvis with the use of 10-0 nonabsorbable monofilament nylon suture and an atraumatic needle. Total ischemic time varied between 30 and 40 min.

Unilateral nephrectomy

Left nephrectomy for the recipient's own kidney was performed under ether anesthesia using an abdominal approach 1 week after the transplantation.

Immunosuppression

Cyclosporin (CyA, Sandimmun, Sandoz, Basel, Switzerland), in a concentration of 50 mg/ml, was dissolved in intralipid (Kabi Vitrum, Stockholm, Sweden) to a concentration of 1, 2, and 3 mg/ml. CyA was administered subcutaneously once a day in a volume of 0.5 ml/100 g of body weight. The diagnosis of acute allograft rejection was made if there was an unexplained rise in serum creatinine over 180 μmol/l and a concordant finding on biopsy. On such occasions additional CyA, at a dose of 5 mg/kg of body weight, was administered subcutaneously for 7 days.

Experimental groups

Subcutaneous administration of CyA at doses of 5, 10, and 15 mg/kg per day in two different protocols was started immediately after completion of the operation. Nine groups of rats were included. In the first five groups, DA kidneys were grafted into WF rats. In the last three groups, transplantations were made from DA-to-DA strain (syngeneic controls). The experimental groups were as follows:

Group 1: 5 mg/kg per day CyA, 0–13 days (allogeneic), $n = 5$
Group 2: 10 mg/kg per day CyA, 0–13 days (allogeneic), $n = 5$
Group 3: 15 mg/kg per day CyA, 0–13 days (allogeneic), $n = 5$
Group 4: 5 mg/kg per day CyA, 0–13 and 21–27 days (allogeneic), $n = 5$
Group 5: 10 mg/kg per day CyA, 0–13 and 21–27 days (allogeneic), $n = 5$
Group 6: 5 mg/kg per day CyA, 0–13 and 21–27 days (syngeneic), $n = 5$
Group 7: 10 mg/kg per day CyA, 0–13 and 21–27 days (syngeneic), $n = 3$
Group 8: 15 mg/kg per day CyA, 0–13 and 21–27 days (syngeneic), $n = 5$
Group 9: Syngeneic controls without CyA treatment, $n = 5$

The diagnosis of acute allograft rejection was made 38 times. Additional CyA at a dose of 5 mg/kg of body weight for 7 days was administered to 16 rats via the subcutaneous route. This occurred with 6 rats in group 1, 14 in group 2, 12 in group 3, 1 in group 4, and 5 in group 5.

Blood sampling

Blood was drawn from the tail tip. Serum creatinine and CyA concentration were measured once a week until the rats were sacrificed.

Determination of CyA levels in recipient blood

Whole blood CyA levels were measured by radioimmunoassay (Sandimmun-Kit, Sandoz, Basel, Switzerland) once a week during the treatment period.

Biopsies

An open kidney biopsy under ether anesthesia utilizing the Biopty (Radioplast, Uppsala, Sweden) instrument with the Biopty-Cut (Radioplast, Uppsala, Sweden) needle was performed at 2, 4, and 8 weeks. The needle was placed parallel to the long axis of the graft. Two cores of tissue, each approximately 15 mm in length, were obtained. The animals were sacrificed 10–12 weeks post-transplantation. All core biopsies and grafts were fixed in buffered formalin and examined histologically following sectioning and staining with hematoxylin-eosin, Masson's trichrome, periodic acid-Schiff, and methyl green-pyronin. All specimens were scored blindly from 0 to 3 (0 indicating no pathological alterations and 3 indicating extreme changes) by two investigators and the score was determined after a consensus had been reached (Table 1). Altogether, 172 biopsies with four different stainings were thus evaluated in this study.

Statistics

For statistical analysis, the Mann-Whitney U-test was used. The results are expressed as mean ± SD and a probability of less than 0.05 was accepted as significant.

Results

Clinical course

The mean serum creatinine levels of the recipient rats – measured separately for the allograft groups receiving CyA for 2 or 3 weeks – and for the syngeneic controls, receiving CyA for 3 weeks, are shown in Fig. 1. After

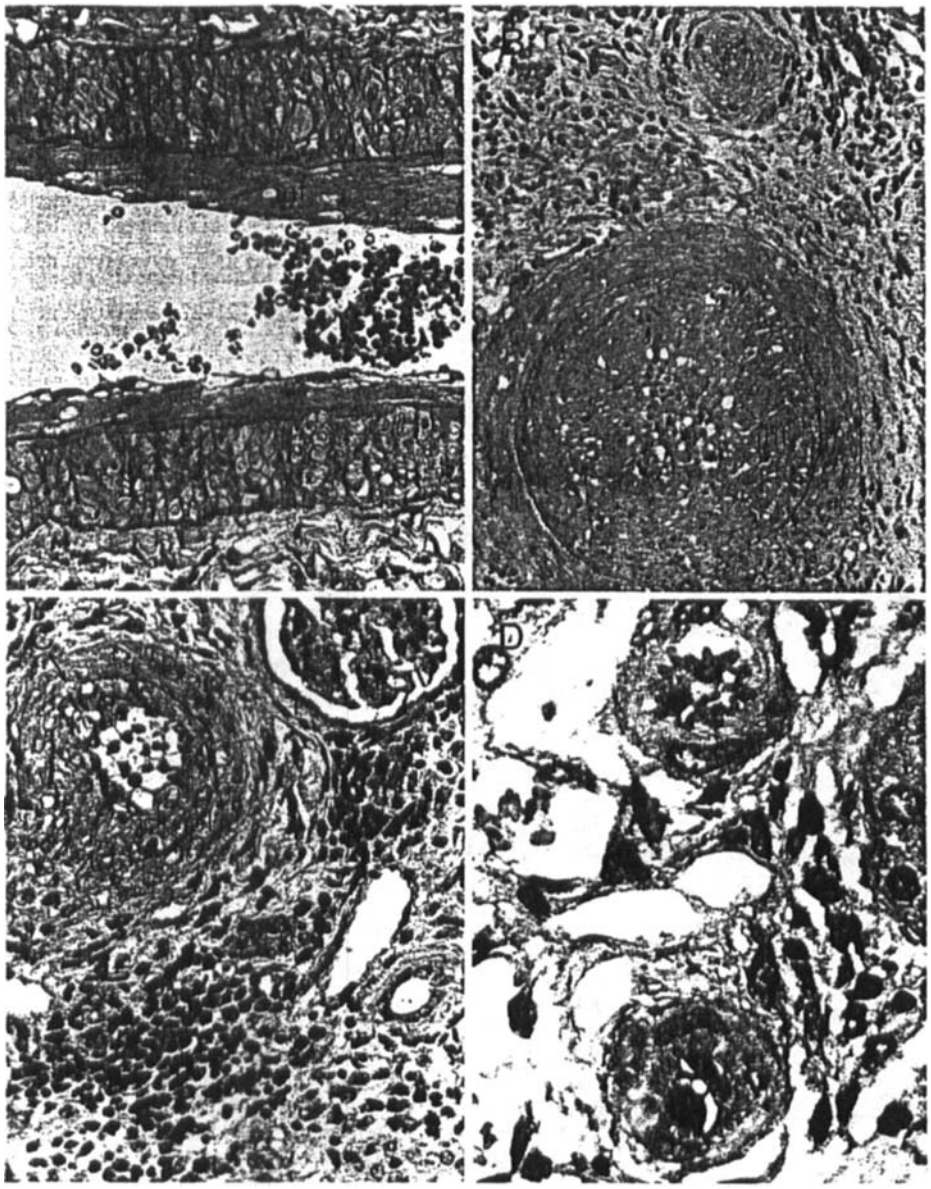


Fig. 3. A–D. Vascular changes in long-surviving DA-to-WF rat renal allografts: **A** longitudinal section of a major vessel demonstrating the intimal *(int)* proliferative response along the vessel wall; **B** the concentric intimal proliferation and occlusion with some foamy vacuolation of a medium-sized artery; **C** intimal proliferation and partially disrupted internal elastic lamina *(iel)*, together with occasional breaks *(arrows)* and parenchymal inflammation (periodic acid-Schiff × 300); **D** endothelial swelling and proliferation in an arteriole (periodic acid-Schiff × 750)

2 weeks of treatment with CyA, all groups of allografted rats demonstrated a slight increase in serum creatinine compared to control values (indicated as a closed box in Fig. 1). When the initial CyA treatment was extended to 3 weeks, the increase in the level of serum creatinine in the allograft recipients was less pronounced. In the syngeneic controls, particularly when employing the highest CyA dose of 15 mg/kg per day, there was a slight initial increase in serum creatinine, indicating acute toxicity, which then declined to the normal control level after CyA was discontinued.

In the 2-week treatment protocol for allografts, the peak values of whole blood CyA concentrations of 1250, 2800, and 3500 µg/l for the 5, 10, and 15 mg/kg per day doses, respectively, were reached 1 week post-transplantation. In the 3-week protocol, similar blood CyA concentrations, demonstrating two different peaks, were observed. In syngeneic controls, blood CyA levels were similar to those reached in the allograft recipients (not shown). One week after discontinuation of the treatment, no CyA was left in the circulation.

Histopathology

In the first biopsies taken during a period of 2–4 weeks post-transplantation, the renal histopathology was dominated, after discontinuation of CyA, by changes characteristic of acute tubulointerstitial rejection of mild to medium intensity. Later, the histopathological features were mainly characteristic of chronic rejection, depending, however, on the duration of the immunosuppressive treatment and on the dosage of the drug.

Figures 2–5 are characteristic of the rats receiving 10 mg/kg per day of CyA for 2 weeks and demonstrate typical histopathology of renal allografts 10–12 weeks post-transplantation, at the moment of sacrifice.

The extensive features were those described previously for chronic rejection and/or for chronic CyA ne-

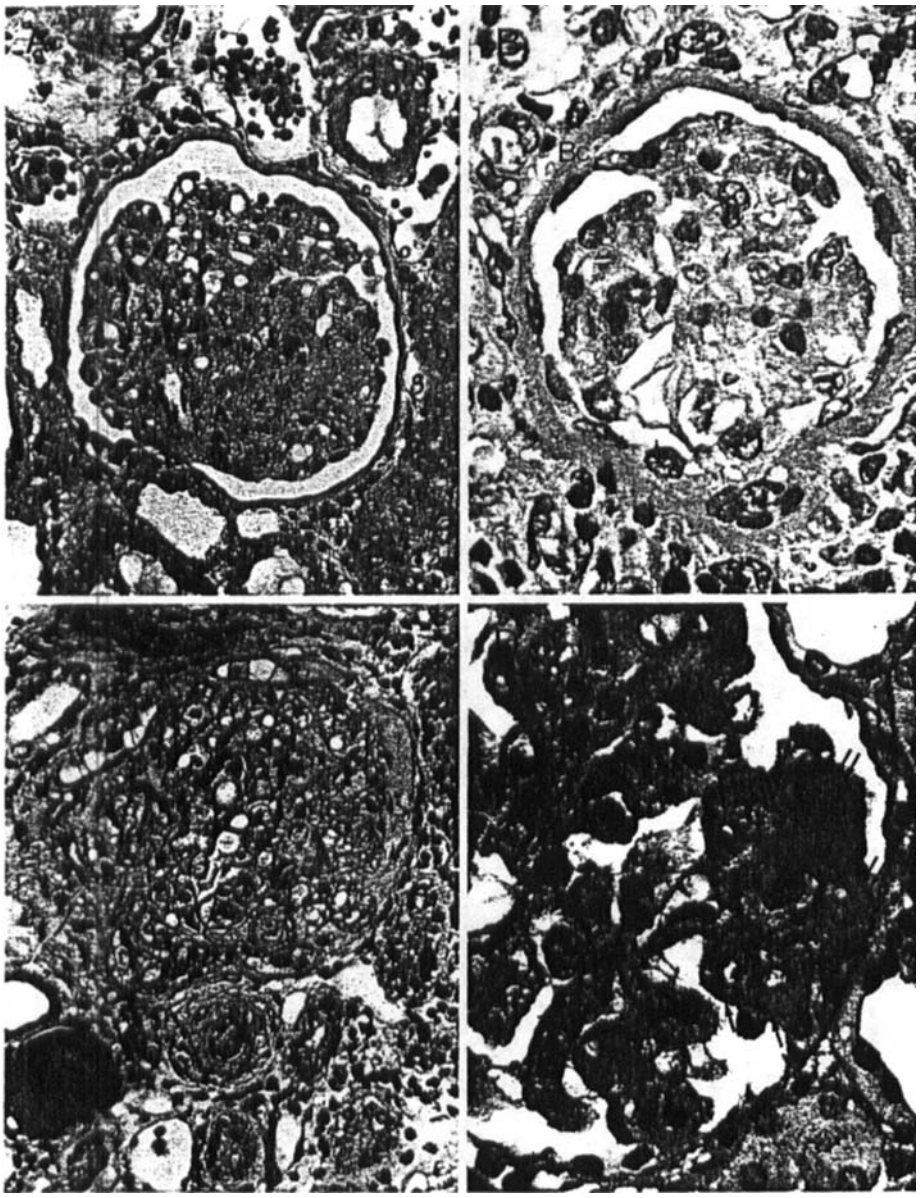


Fig. 4. A–D. Glomerular changes in long-surviving DA-to-WF rat renal allografts:
A incipient;
B pronounced; and
C extreme alterations;
D basement membrane thickening and doubling *(arrows)* and occlusion of the glomerular capillaries *(double arrows)*
(**A, C** periodic acid-Schiff ×300; **B, D** periodic acid-Schiff ×750)

phrotoxicity in human allograft biopsies, i.e., an ongoing inflammation in the graft, particularly in the vicinity of the major vessels and also extending into the tubulointerstitial space, interstitial fibrosis, glomerular sclerosis, a strong vascular intimal response, sometimes leading to a nearly complete occlusion of the vessels, and tubular vacuolization and atrophy.

Quantitation of the histological alterations: differentiation from cyclosporin nephrotoxicity

The most prominent histological alterations in these specimens are quantitated in Tables 2 and 3. At the end of the observation period, 10–12 weeks post-transplantation, prominent inflammation was present in the allografts, particularly in the 2-week treatment groups. The inflammation consisted of mononuclear cells of both lymphocytic and monocytic types, and there was a strong pyroninophilic component in the inflammatory infiltrate. Graft fibrosis was also evident. In the glomeruli, the most striking features were mesangial matrix increase and basement membrane thickening, leading to glomerular sclerosis. A very prominent intimal proliferative response was recorded in the allograft arteries, particularly in the arcuate and interlobular arteries and extending to the arterioli. Concomitantly, these vessels became obliterated. In the tubular compartment, epithelial vaculation, epithelial atrophy, and basement membrane thickening were the most dominant features.

Which of these parameters were due to chronic rejection and which were due to CyA nephrotoxicity or to other unidentified factors, such as the operation per se, was investigated by comparing the allografts to syngeneic grafts in corresponding dosage groups.

Tables 2 and 3 demonstrate the statistical significance in the scores of the above-mentioned alterations in the allografts compared to the syngeneic controls. There was a

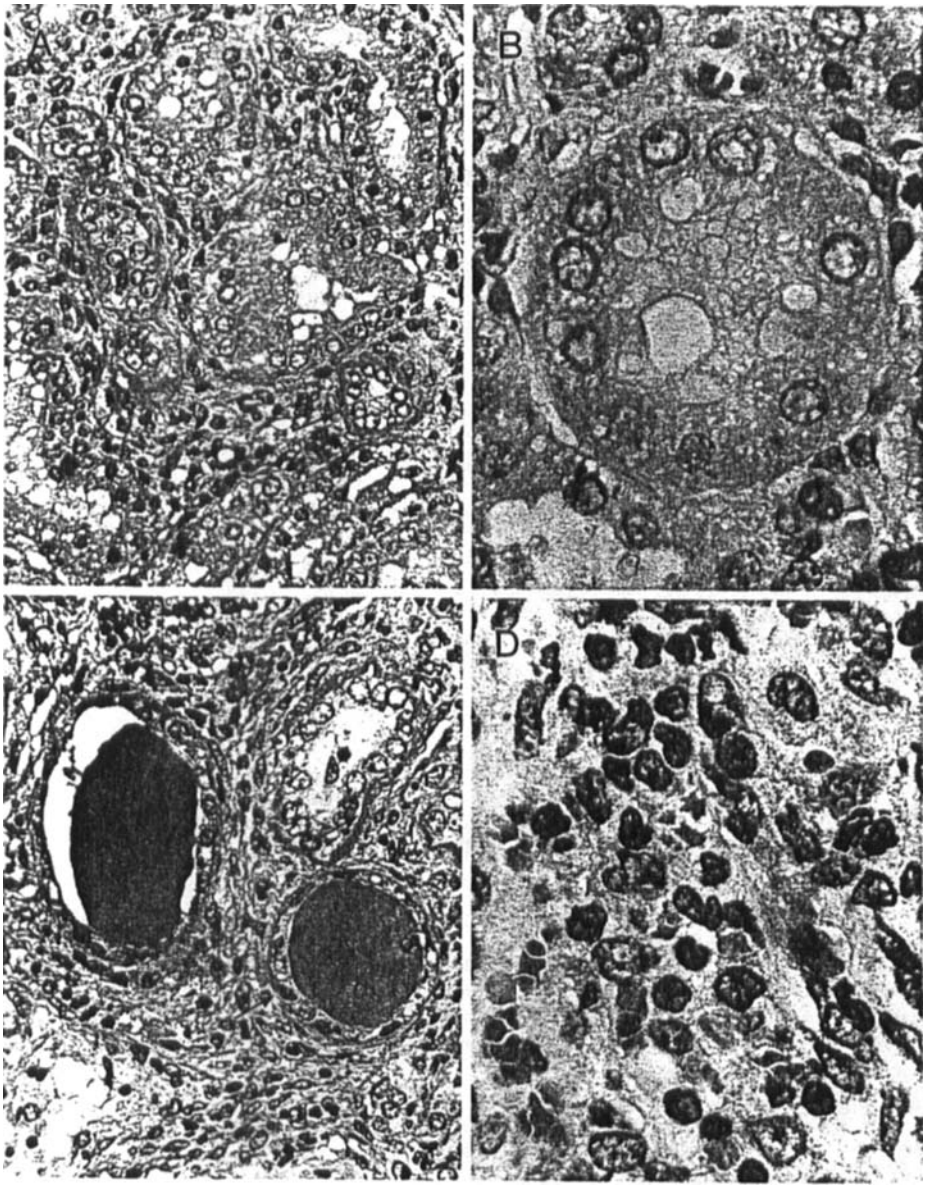


Fig. 5. A–D. Tubular and interstitial changes in long-surviving DA-to-WF rat renal allografts:
A advanced tubulopathy, swelling, and vacuolation in tubuli, thickening of basement membrane *(arrow)*, and interstitial inflammation and fibrosis;
B swollen, vacuolated tubules;
C tubuli with atrophic epithelium and casts, thickening of the basement membranes *(arrows)*, and interstitial inflammation;
D a detail of interstitial inflammatory response demonstrating mostly lymphocytes and macrophages (**A, C** periodic acid-Schiff ×300; **B, D** periodic acid-Schiff ×750)

significantly higher level of interstitial and perivascular inflammation in the allografts than in the syngeneic grafts, particularly when considering the pyroninophilic component of the inflammatory cells. Fibrosis, however, was present both in the allografts and in the syngeneic grafts.

In the glomeruli, the increase in the mesangial matrix contents, basement membrane thickening, and duplication and glomerular sclerosis were all present in the allografts in a significantly higher intensity than in the syngeneic controls.

The most striking differences were observed in the graft vasculature. The allograft arterial tree demonstrated a very significant intimal proliferative response and obliteration of the vessels, as compared to the syngeneic controls, where practically no vascular alterations were recorded.

To clear contrast in the tubular compartment, epithelial vacuolation, epithelial atrophy, and basement membrane thickening were recorded in approximately similar intensities both in the allografts and in the syngeneic controls.

An additional control group was created by comparing CyA-treated syngeneic transplants with syngeneic transplants not receiving CyA. This result is shown in Table 2. Only very minor histological alterations were seen in the syngeneic controls not receiving CyA.

Sequence of the development of the histopathological lesions

From what has been described above, it appears that the main features of chronic rejection in our rat allograft model are the following: persistent interstitial and perivascular inflammation, a strong pyroninophilic component in the inflammatory infiltrate, glomerular sclerosis, and a very strong, occlusive intimal response in the allograft vascular tree.

The sequence of the development of these alterations was quantitated from the biopsies performed on the trans-

Table 2. Effect of cyclosporin (CyA) dose on the histopathology of DA-to-WF rat renal allografts and of DA-to-DA syngeneic grafts: 2-week treatment of allograft recipients. Arbitrary scale from 0 (no changes) to 3 (maximal changes)

	CyA dose (mg/kg per day)[a]						Syngeneic graft without CyA[b]
	Allograft			Syngeneic graft			
	5	10	15	5	10	15	
Interstitium							
Inflammation	1.10	1.50*	1.80**	0.50	0.50	0.50	0.20
Pyroninophilic cells (%)	21.2*	28.0*	28.0**	1.00	2.00	2.60	0.00
Fibrosis	1.00	1.50	1.40*	0.60	1.00	0.70	0.30
Glomeruli							
Mesangial matrix increase	1.70*	2.20*	2.10*	0.90	0.67	1.00	0.70
Capillary basal membrane thickening	0.90*	1.70*	1.40*	0.20	0.00	0.40	0.30
Sclerosis	0.70	1.60*	1.00*	0.00	0.00	0.00	0.10
Vessels							
Intimal proliferation	1.70**	2.20*	2.20**	0.00	0.00	0.20	0.00
Obliteration	1.30*	1.50*	1.60*	0.00	0.00	0.00	0.00
Tubuli							
Epithelial vacuolation	0.20	0.80	1.10	0.20	0.67	0.80	0.00
Epithelial atrophy	0.60	0.80	0.70	0.40	1.00	0.40	0.40
Capillary basal membrane thickening	0.50	0.60	0.60	0.40	0.50	0.20	0.10

* $P < 0.05$, ** $P < 0.01$ (Mann-Whitney U-test) to CyA-treated syngeneic controls
[a] Allogeneic rats received CyA for 2 weeks, syngeneic rats for 3 weeks
[b] DA-DA syngeneic grafts without CyA

plants at 2, 4, 8, and 12 weeks post-transplantation. This is shown in Figs. 6–10.

The first histopathological change recorded in these transplants was perivascular and interstitial inflammation (Fig. 6). The inflammatory response was strongest in the allografts receiving 2 weeks of CyA immunosuppression, followed by the allografts receiving CyA for 3 weeks; there was also a slight but significant inflammatory response in the syngeneic controls. In the 2-week allograft treatment group, the inflammation commenced shortly after the discontinuation of immunosuppression and continued on approximately this level throughout the observation period. In the 3-week allograft treatment group, the inflammation commenced later but reached equivalent intensity at the end of the observation period (not shown).

Table 3. Effect of cyclosporin (CyA) dose on the histopathology of DA-to-WF rat renal allografts and of DA-to-DA syngeneic grafts: 3-week treatment of allograft recipients. Arbitrary scale from 0 (no changes) to 3 (maximal changes)

	CyA dose (mg/kg per day)[a]			
	Allograft		Syngeneic graft	
	5	10	5	10
Interstitium				
Inflammation	1.20	1.30	0.50	0.50
Pyroninophilic cells (%)	16.0*	21.0*	1.00	2.00
Fibrosis	0.80	1.40	0.60	1.00
Glomeruli				
Mesangial matrix increase	1.50	1.80*	0.90	0.67
Capillary basal membrane thickening	1.10*	1.00	0.20	0.00
Sclerosis	0.60	0.20	0.00	0.00
Vessels				
Intimal proliferation	0.70*	1.40*	0.00	0.00
Obliteration	0.30	1.10	0.00	0.00
Tubuli				
Epithelial vacuolation	0.90	1.00	0.20	0.67
Epithelial atrophy	0.50	0.80	0.40	1.00
Capillary basal membrane thickening	0.10	0.40	0.40	0.50

* $P < 0.05$ (Mann-Whitney U-test)
[a] Allogeneic rats received CyA for 3 weeks, syngeneic rats for 3 weeks

A striking feature within the inflammatory infiltrate in the allografts but not in the syngeneic controls was a strong pyroninophilic component in the inflammatory leukocytes (Fig. 7). Pyroninophilia, indicating high amounts of RNA in the inflammatory leukocytes and, indirectly, the proliferative response of the inflammatory cells, was stronger and developed earlier in the allografts immunosuppressed with CyA for 2 weeks than in those immunosuppressed for 3 weeks (not shown).

The intimal thickening developed within the graft vasculature after the onset of inflammation (Fig. 8). In the grafts immunosuppressed for 2 weeks, incipient intimal changes were already evident at 4 weeks post-transplantation; they were quite clear at 8 weeks and culminated at 12 weeks, at the time of sacrifice. In the allografts immunosuppressed for 3 weeks, the vascular changes were recorded later (not shown). No vascular changes were recorded in the syngeneic controls. When the vascular changes were quantitated using vessel obliteration as the test parameter, the changes were similar (Fig. 9).

Glomerular sclerosis developed even later (Fig. 10): it was evident at 8 weeks post-transplantation in the allografts immunosuppressed for 2 weeks and it was strongest at 12 weeks, at the time of sacrifice. In the allografts immunosuppressed for 3 weeks, only nonsignificant glomerular sclerosis was observated at 12 weeks post-transplantation (not shown). No glomerular changes were recorded in the syngeneic controls.

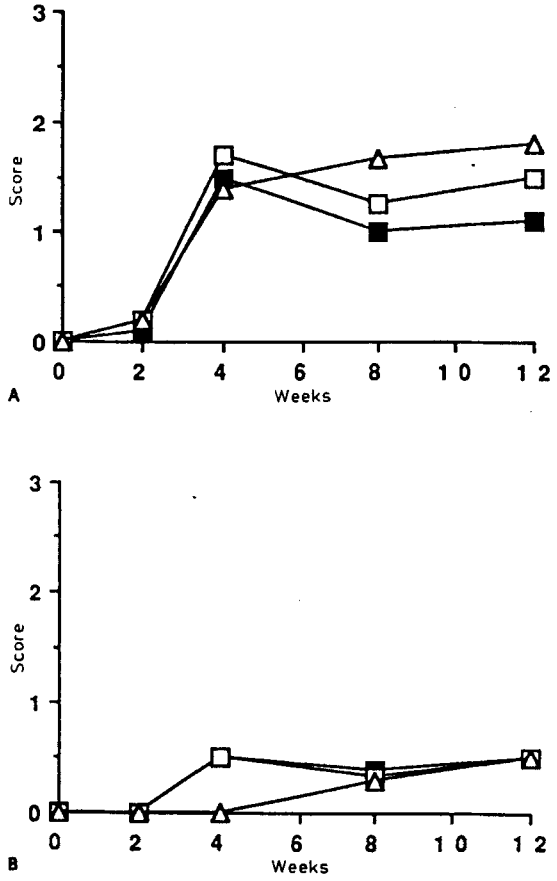


Fig. 6. A, B. Intensity of interstitial inflammation: **A** in rat renal allografts and **B** in syngeneic grafts immunosuppressed with 5, 10, or 15 mg/kg per day of CyA. All points represent the mean values of five separate transplants. ■ 5 mg/kg; □ 10 mg/kg; △ 15 mg/kg

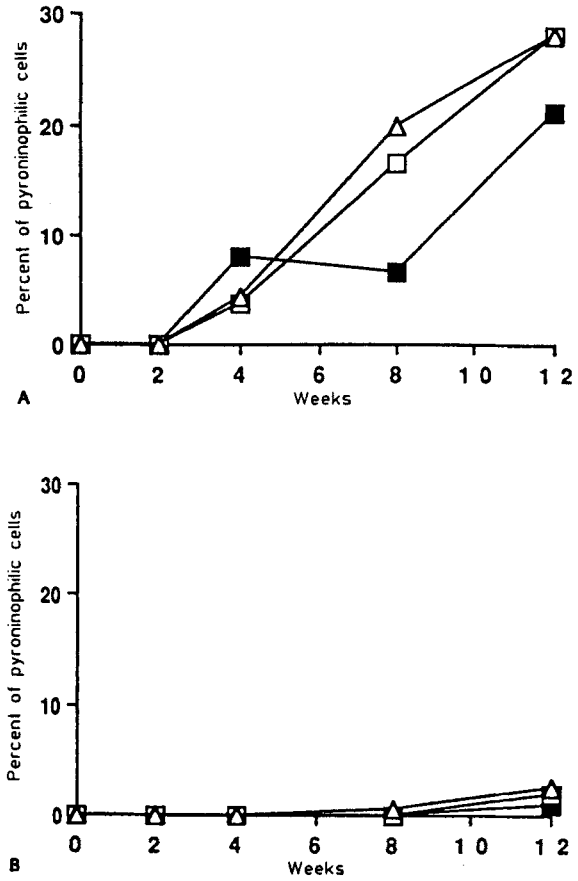


Fig. 7 A, B. Extent of pyroninophilia (%) of inflammatory mononuclear cells: **A** in rat renal allograft and **B** in syngeneic grafts immunosuppressed with 5, 10, or 15 mg/kg per day of CyA. All points represent the mean values of five separate transplants. Symbols as in Fig. 6

Discussion

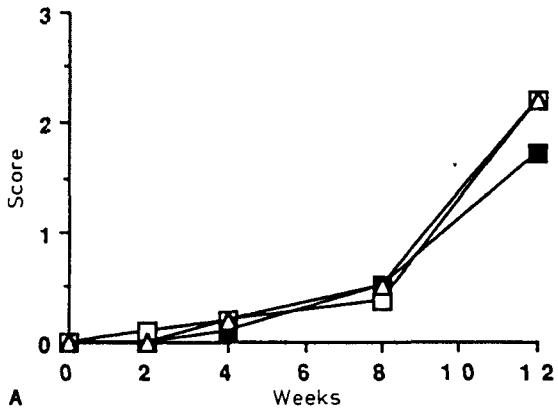
The first purpose of this communication was to determine, using an experimental rat model previously employed for allograft "tolerance" [15, 16, 25], which light microscopic lesions in long-surviving renal allografts are attributable to chronic rejection, which to chronic cyclosporin nephrotoxicity, and/or which to the operative procedure per se.

The histological lesions observed in our long-surviving renal allografts are in many ways similar to those observed previously in human renal allografts during the late post-transplantation period and attributed to chronic rejection [5, 22]. Our histological lesions are also similar to the experimental observations of Porter et al. [21] on long-term renal allografts in dogs and of White et al. [26] in rat renal allografts transplanted across minor barriers. The clinical features in our transplants are equally compatible with the histopathological observations: deterioration of graft function developed concomitantly with the development of histopathological alterations. We therefore consider it likely that some of these alterations must be attributable to chronic rejection, although the possible influence of CyA and the operative procedure per se in the generation of these changes must first be excluded.

This was achieved in our study by comparing allografts to syngeneic transplants receiving identical dosages of CyA and by comparing the less intensively immunosuppressed allografts to the more intensively immunosuppressed ones.

Persistent inflammation, a strong pyroninophilic component in the inflammatory infiltrate, glomerular mesangial matrix increase, basement membrane thickening, sclerosis of the glomeruli, and – by far the most – intimal proliferation and vascular obliteration were significantly more prominent in the allografts than in the syngeneic controls. Virtually no histological alterations were observed in syngeneic controls not receiving CyA. Therefore, we consider this group of histopathological alterations to be mainly due to chronic rejection. In contrast, interstitial fibrosis and tubular alterations, i.e., epithelial vacuolation and atrophy, were not significantly different in the controls. Thus, the latter group of changes were apparently mainly due to CyA treatment and/or to other transplantation-related factors. From our results it is, however, also evident that these "perioperative" factors also contributed, to some extent, to inflammation (but not to pyroninophilia) and to glomerular mesangial matrix increase (but not to glomerular sclerosis) that is, to some of the alterations described here as characteristic of chronic rejection.

Our finding that the morphological alterations of chronic cyclosporin toxicity (with the exception of arteri-



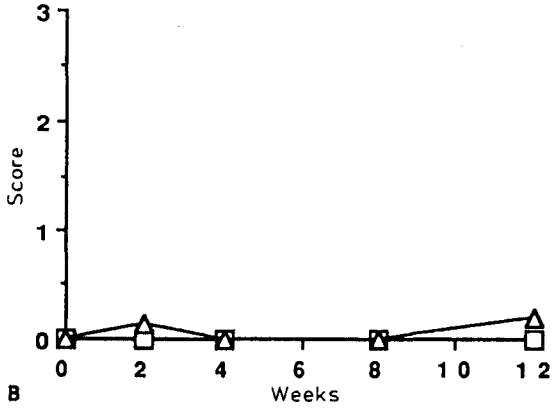
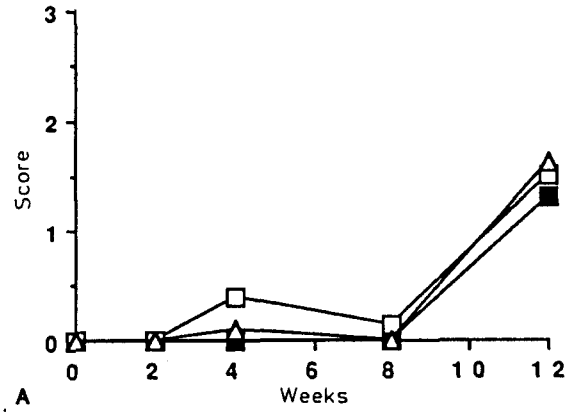


Fig. 8 A, B. Vascular intimal response: **A** in rat renal allografts and **B** in syngeneic grafts immunosuppressed with 5, 10, or 15 mg/per day of CyA. All points represent the mean values of five separate transplants. Symbols as in Fig. 6



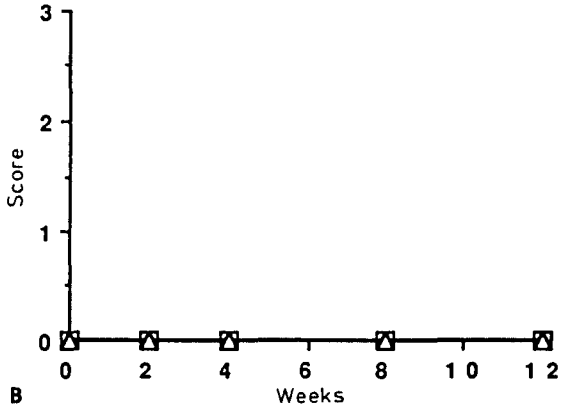


Fig. 9 A, B. Vascular obliteration: **A** in rat renal allografts and **B** in syngeneic grafts immunosuppressed with 5, 10, or 15 mg/kg per day of CyA. All points represent the mean values of five separate transplants. Symbols as in Fig. 6

olar manifestations) are primarily tubulointerstitial is compatible with previous experimental and clinical data. Rats receiving comparable doses (5 mg/kg per day) of CyA for 21–32 weeks developed primarily atrophic tubular alterations and interstitial fibrosis with some segmental glomerular lesions in their native kidneys. Only focal lymphocytic infiltration and no vascular changes were present [1]. When a higher dosage of 20–25 mg/kg per day was used for 28–84 days, the tubular vacuolization, inclusions, and atrophy were more prominent, as was interstitial inflammation and fibrosis. In addition, arteriolar changes were seen. Again, the major vessels remained unchanged [11, 23]. To our knowledge no single study has investigated the long-term effects of CyA nephrotoxicity in experimental syngeneic grafts.

A 14-day oral administration of CyA at a dose range of 5–20 mg/kg per day results in a significant increase in the blood pressure of the rat [12]. This raises the possibility that some of these vascular lesions were due to hypertension. It is, however, unlikely that the vascular changes we observed (intimal thickening, obliteration of the lumen) were due to hypertension since practically none of these alterations were observed in syngeneic cyclosporin-treated controls.

The most notable changes in the native kidneys of human subjects immunosuppressed with CyA for reasons other than a kidney transplant are also mainly tubulointerstitial [19, 20]. Why arteriolar changes, described by Mihatsch et al. [18] as characteristic of chronic CyA nephrotoxicity, were less evident in our transplants may be due to the smaller dose and to the shorter treatment period in our study.

Our second goal was to obtain more insight into the sequence of the generation of the chronic lesions and, thereby, to gather information on the pathogenesis of chronic kidney allograft rejection.

It has previously been demonstrated by several groups [7, 13–15, 25] that short-term CyA treatment for recipient rabbits and rats, sometimes after an initial (subacute) episode of inflammation, leads to long-term survival of a large fraction of several types of allografts. Consequently, these rabbits and rats display immunological nonreactivity ("tolerance") to the donor strain. If the treatment period with CyA is extended from 2 to 3 weeks, the fraction of surviving allografts increases correspondingly [16]. In this study we show that, depending on the dose and duration of CyA treatment, some of these grafts develop an attenuated decline in graft function with histopathological alterations similar to those previously reported for chronic rejection in humans.

Demetris et al. [6], Foegh [9], and Billingham [2] have recently reviewed the histopathology of chronically re-

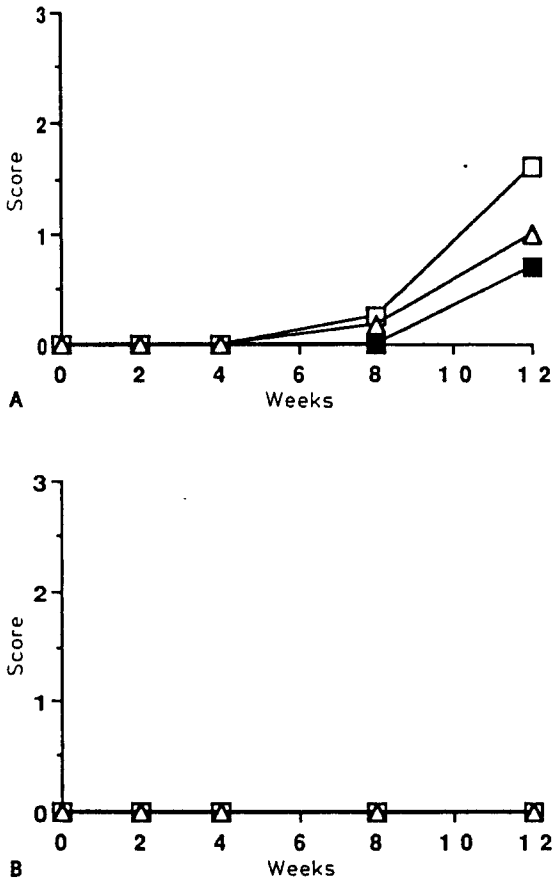


Fig. 10 A, B. Glomerular sclerosis: **A** in rat renal allografts and **B** in syngeneic grafts immunosuppressed with 5, 10, or 15 mg/kg per day of CyA. All points represent the mean values of five separate transplants. Symbols as in Fig. 6

jecting human kidney, liver, and heart allografts on the basis of biopsy or autopsy specimens. According to their interpretation, the central feature of all chronically rejecting transplants is an intimal obliterative response. Although some interorgan differences do exist [6], the basic features of the intimal response are the following: persistent periarterial inflammation, influx of the intima by monophagocytes and smooth muscle cells, a gradual disappearance of nuclei from the media, and a grossly intact internal and external elastic lamina with occasional breaks in the internal. These vascular changes, which were previously attributed to chronic rejection of human allografts, are virtually identical in our experimental rat renal allografts.

Chronologically, the histopathological alterations may be divided into two largely overlapping phases: (1) an acute episode of inflammation appearing shortly after discontinuation of CyA treatment and (2) gradually increasing parenchymal changes in the graft.

An acute inflammatory response, with characteristics of an acute, cellular tubulointerstitial rejection, seems to be a prerequisite for the generation of the chronic changes. The strongest early inflammatory episodes were recorded in the allografts immunosuppressed for 2 weeks rather than for 3 weeks. Consequently, the chronic changes were more pronounced. A distinct pyroninophilic component in the inflammatory response was recorded in the allografts but not in the syngeneic grafts. Thus suggests that the stronger the lymphocyte activation is, the more prone the allograft is to develop chronic rejection.

It is, however, likely that this may not be the only pathogenetic mechanism contributing to the generation of a chronic lesion. Human studies, mainly retrospective, suggest that in addition to a high incidence, or severity, of acute episodes of rejection, longer ischemic times may also contribute to the development of chronic rejection [3, 10]. Of the immunological factors, antibodies to class I antigens may be more important than antibodies to class II antigens [24]. These possibilities may now be experimentally tested by our rat model.

It is also possible that CyA, together with an acute rejection episode, may induce additional damage to the graft. In the 2-week allograft group, the extent of chronic damage, as quantitated with serum creatinine, was in reverse relation to the CyA dose (and concentration), and somewhat more vascular obliteration was seen using a higher rather than lower CyA dose.

Taken together, we believe that rat renal allografts immunosuppressed for a short period with CyA offer a new experimental model to study chronic renal allograft rejection in the inbred rat. Such transplants make it possible for the first time to investigate the cellular and molecular mechanisms of chronic rejection in the kidney allograft and may provide experimental evidence of its etiology and treatment.

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References

1. Bäckman L, Sundelin B, Bohman S-O (1990) Chronic renal lesions in rats after long-term treatment with cyclosporine A. Transplant Proc 22: 133–134
2. Billingham ME (1987) Cardiac transplant atherosclerosis. Transplant Proc 19 [Suppl 5]: 19–25
3. Cecka MJ, Terasaki PI (1989) Early rejection episodes. In: Terasaki PI (ed) Clinical transplants 1989. UCLA Tissue Typing Laboratory, Los Angeles, pp 425–434
4. Cecka JM, Corcoran S, Takemoto S, Cho Y, Mickey MR, Terasaki PI (1989) The UNOS scientific renal transplant registry. In: Terasaki PI (ed) Clinical transplants 1989. UCLA Tissue Typing Laboratory, Los Angeles, pp 1–8
5. Croker BP, Salomon DR (1989) Pathology of the renal allograft. In: Tisher CC, Brenner BM (eds) Renal pathology, vol 2. Lippincott, Philadelphia, pp 1518–1551
6. Demetris AJ, Zerbe T, Banner B (1989) Morphology of solid organ allograft arteriopathy: identification of proliferating intimal cell populations. Transplant Proc 21: 3667–3669
7. Engemann R, Ulrichs K, Thiede A, Müller-Ruchholtz W, Hamelmann H (1982) Value of a physiological liver transplant model in rats. Transplantation 33: 566–568
8. Fisher B, Lee S (1965) Microvascular surgical techniques in research, with special reference to renal transplantation in the rat. Surgery 58: 904–913
9. Foegh ML (1990) Chronic rejection-graft arteriosclerosis. Transplant Proc 22: 119–122

10. Foster MC, Morgan AG, Rowe PA, Burden RP, Dennis MJS, Blamey RW (1990) Characteristics of cadaveric renal allograft recipients developing chronic rejection. Ann R Coll Surg Engl 72: 23–26
11. Gillum DM, Truong L, Tasby J, Migliore P, Suki WN (1988) Chronic cyclosporine nephrotoxicity. Transplantation 46: 285–292
12. Golub MS, Lustig S, Berger ME, Lee DB (1989) Altered vascular responses in cyclosporine-treated rats. Transplantation 48: 116–118
13. Green CJ, Allison AC (1978) Extensive prolongation of rabbit kidney allograft survival after short-term cyclosporin-A treatment. Lancet I: 1182–1183
14. Hall BM, Gurley KE, Pearce NW, Dorsch SE (1989) Specific unresponsiveness in rats with prolonged cardiac allograft survival after treatment with cyclosporine. II. Sequential changes in alloreactivity of T cell subsets. Transplantation 47: 1030–1033
15. Homan WP, Fabre JW, Morris PJ (1979) Nature of the unresponsiveness induced by cyclosporin-A in rats bearing renal allografts. Transplantation 28: 439–441
16. Lim SML, White DJG, Calne RY (1988) Cyclosporine coverage during the risk period leads to 100% long-term graft acceptance in the rat. Transplant Proc 20 [Suppl 3]: 1013–1015
17. Mihatsch MJ, Thiel G, Spichtin HP, Oberholtzer M, Brunner FP, Harder F, Olivieri V, Bremer R, Ryffel B, Stöcklin E, Torhorts J, Gudat F, Zollinger HU, Loertscher R (1983) Morphological findings in kidney transplants after treatment with cyclosporine. Transplant Proc 15: 2821–2835
18. Mihatsch MJ, Ryffel B, Gudat F, Thiel G (1989) Cyclosporine nephrotoxicity. In: Tisher CG, Brenner BM (eds) Renal pathology with clinical and functional correlations. Lippincott, Philadelphia, pp 1555–1586
19. Myers BD (1986) Cyclosporine nephrotoxicity. Kidney Int 30: 964–974
20. Palestine AG, Austin HA, Balow JE, Antonovych TT, Sabnis SG, Preuss HG, Nussenblatt RB (1986) Renal histopathologic alterations in patients treated with cyclosporine for uveitis. N Engl J Med 314: 1293–1298
21. Porter KA, Calne RY, Zukoski CF (1964) Vascular and other changes in 200 canine renal homotransplants treated with immunosuppressive drugs. Lab Invest 13: 809–824
22. Sanfilippo F (1990) Renal transplantation. In: Sale GE (ed) The pathology of organ transplantation. Butterworths, Boston, pp 51–101
23. Simpson JG, Saunders NJ, Thompson KJ, Whiting PH (1988) Chronic renal damage caused by cyclosporine. Transplant Proc 20: 792–799
24. Terasaki PI, Park MS, Takemoto S, Cecka JM, Clark B, Corcoran S, Cicciarelli J, Barbetti A, Yuge J, Carnahan E (1989) Overview and epitope matching. In: Terasaki PI (ed) Clinical transplants 1989. UCLA Tissue Typing Laboratory, Los Angeles, pp 499–516
25. White DJG, Rolles K, Ottawa T, Turell O (1980) Cyclosporin-A induced long-term survival of fully incompatible skin and heart grafts in rats. Transplant Proc 12: 261–265
26. White E, Hildeman WH, Mullen Y (1969) Chronic kidney allograft reactions in rats. Transplantation 8: 602–617