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Arteriolosclerosis of the human renal allograft: morphology, origin, life history and relationship to cyclosporine therapy

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Summary. In the decade 1979–1988, 658 biopsies were collected from 568 cadaveric renal allografts. In 118 grafts a non-proliferative insudative vasculopathy (IVA) was found in afferent vessels. Immunosuppression was based on azathioprine (AZA) or on cyclosporin A (CsA), from 1983. The prevalence and extent of IVA has increased significantly since 1984. Light microscopy showed fibrinoid and hyaline masses of varying extent; transmural insudative "knobs", intimal oedema with metachromasia, and microthrombosis were also seen with CsA. The ultrastructure of the insudates was unremarkable but CsA grafts displayed early oedema and hypergranulation of endothelial cells with a disarray of smooth muscle cell (SMC) microfibrils, and pronounced degenerative changes of SMC. Rebiopsy showed stationary IVA in AZA grafts and progression in one-half of CsA-treated patients. Nephrectomy specimens revealed, however, a marked predominance of late rejection endarteritis; in only 3 cases was IVA and/or microthrombosis the possible cause of nephrectomy. The mean donor age was higher in severe IVA in CsA grafts and the mean post-transplantation interval at the time of diagnosis of IVA was significantly shorter in CsA-treated patients. No important differences in cumulative graft survival were seen between grafts with absent, moderate or severe IVA. Unused cadaveric donors' kidneys of comparable age exhibited normal arterioles or a slight focal insudative or hyaline lesion.

Key words: Kidney allotransplantation – Renal biopsy – Cyclosporin A toxicity – Graft arteriolosclerosis – Renal ultrastructure

Introduction

Vascular rejection lesions are the prime cause of renal allograft failure. Both the early form, obliterative arterio-arteriolopathy (OA) (Rossmann et al. 1970; Callard et al. 1975), and the late variant, rejection endarteritis (EA) (Porter 1974; Rossmann and Jirka 1979), are progressive and irreversible, and differ in morphology, topography, clinical course and final outcome. During 1983–1985 the conventional azathioprine (AZA)-based immunosuppression was gradually replaced by cyclosporin A (CsA, Sandimmune) or by CsA-AZA combinations in our centre, and since this period more frequent and prominent arteriolar changes similar to hyaline arteriolosclerosis have been observed by needle biopsy. This insudative vasculopathy (IVA) differs from conventional rejection lesions and has been interpreted as a possible late consequence of toxic damage by CsA (Mihatsch et al. 1986, 1988; Sommer et al. 1986; Myers et al. 1988).

The present study is based on the revision of 658 graft core biopsies obtained in 1979–1988 with the analysis of 118 selected grafts expressing various degrees of IVA. Its main goal was to assess the influence of CsA therapy on the prevalence, morphology and consequences of IVA. Although this change lacks specific structures and signs some details of morphology and graft life history diverge from those usual before the introduction of CsA. The prognostic significance of IVA is less ominous than that of classical rejection vascular damage, especially OA.

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Materials and methods

Between 1979 and 1988, 568 renal allotransplantations (Tx) were performed at the IKEM Transplant Centre. From these 658 biopsies were collected. In 217 Tx CsA had been used for immunosuppression (1983–1988). In the biopsies of 118 grafts from 116 patients IVA was observed and 48 additional, previous or subsequent biopsy samples were available for comparison. The immunosuppression was based on CsA in 62 and on AZA in 56 grafts with IVA; the latter group also includes 11 grafts biopsied within the given decade but transplanted earlier (1972–1978). Detailed clinical data were available in 57 and 54 cases. Excised grafts were histologically examined in 42 patients, 22 after AZA- and 20 after CsA-

based therapy. The comparative pre-Tx group comprises 37 previously examined non-used cadaver donor kidneys.

Conventional immunosuppression was carried out with AZA (about 2 mg/kg per day) and prednisone (100 mg/day, since 1983 30 mg/day in the early post-Tx period, with gradual lowering to 10–15 mg/day). The CsA treatment scheme was modified repeatedly: the acceptable range of RIA-determined blood levels (450–900 ng/ml in 1983–1985) was gradually restricted to 190–380 ng/ml from 1987. CsA was combined with prednisone, and from 1986 with AZA in all patients. Rejection episodes were treated by i.v. methylprednisolone and by polyclonal or monoclonal antilymphocyte antibody. The conversion of CsA- to AZA-based therapy was usually done 12 months post Tx; in 15 patients the conversion took place earlier. Needle biopsy was indicated primarily in order to examine an unexplained reduction of graft function, less frequently to document proteinuria and urine cellularity (chiefly in late biopsies of AZA-treated Tx). The cumulative graft survival (Cutler and Ederer 1958) was assessed in two patient groups: the first comprised 21 AZA- and 43 CsA-treated patients with Tx between 1983 and 1988, while the second one was based on 23 selected pairs of comparable age, sex and post-Tx interval. One member of each pair had IVA (23 severe) while in the second IVA was lacking. The statistical evaluation was performed in an EC 1032 computer with BDMP programmes (namely BDMP 7D and 1 L).

For light microscopy the tissue was fixed in 10% buffered formalin and paraffin sections were stained with haematoxylin and eosin (H & E), blue trichrome or chromotrope-aniline blue (CAB), acid fuchsin-orange G (AFOG), periodic acid-Schiff, methenamine-silver (PASM), toludine blue or azur, and aldehyde-fuchsin. Light microscopic changes were assessed independently by two trained pathologists. The electron microscopy was done after fixation in 2.5% buffered glutaraldehyde with postfixation in 2% buffered OsO_4, and embedding in Vestopal W. Uranyl acetate- and Pb citrate-contrasted ultrathin sections were examined under Tesla BS 613, JEM 100 B and JEM 200 CX microscopes. The immunofluorescence microscopy was performed on cryostat sections of unfixed frozen tissue after a direct one-step incubation with FITC-labelled swine antibodies specifically reacting with human gamma, alpha and mu heavy chains, C 3, and fibrinogen-related products (swine anti-human IgG, IgA, IgM, C 3, and Fbg; Sevac Institute, Prague).

In the semiquantitative evaluation two main categories of IVA were distinguished: firstly, moderate (non-stenosing), and secondly, stenosing to obliterative lesion. This latter group comprised arterioles with greater than 50% luminal stenosis by PAS- and/or trichrome-stained fibrinoid or hyaline masses (Fig. 1). Transmural insudates with segmental defects of the media and peripheral protrusion of insudates were always classified as severe. The classification was based on the most damaged arterioles in a given tissue sample and about 30–40 sections per biopsy were inspected. Juxtaglomerular (JGL) granulation was revealed by the PASM method. In semiquantitative assessment JGL areas without or with sparse small granules were scored as 0 and those with uniform small granules as +. The finding of abundant polymorph granules with well-marked clustering and/or expansion of granular cells into the adjacent segment of vas afferens was given 2+. In unequal granulation the most severely involved glomeruli (3 at least) were taken as representative.

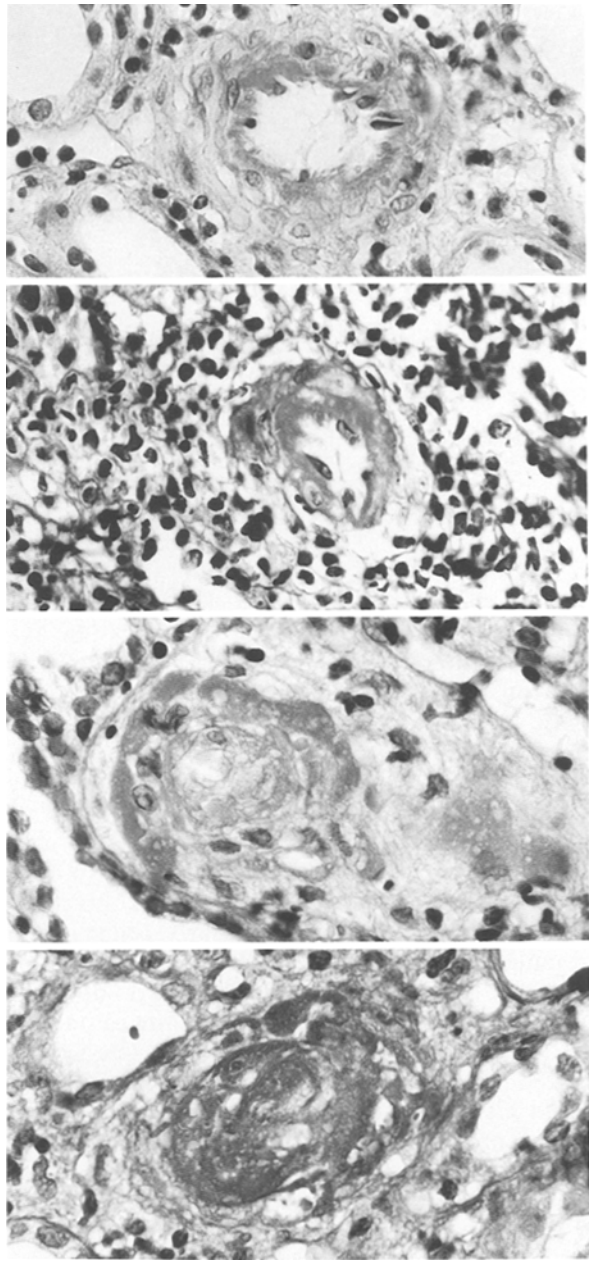


Fig. 1. Light microscopy of insudative vasculopathy (IVA) in afferent arterioles. *Upper pair*: Moderate and medium-degree IVA. A non-stenosing insudate is seen in the top arteriole stained with haematoxylin and eosin (H & E). A slight luminal stenosis is shown in the upper centre vessel stained with chromotrope-anilin blue (CAB). *Lower pair*: Severe IVA with obliterative transmural hyaline and fibrinoid masses (lower centre, H & E; bottom, CAB). Note multiple fibrinoid protrusions – "knobs", especially in the upper and left segments of the lower centre arteriole. All parts, × 520

Results

The characteristic examples of IVA are shown in Fig. 1. IVA typically involved cortical afferent arterioles and end segments and branchings of the interlobular vessels. Its characteristic morphological findings were hyaline and fibrinoid deposits best visualized by PAS and trichrome or CAB, the extent of fibrinoid by AFOG. A second prominent, though inconstant, feature was oedematous intimal thickening of reticular or foamy appearance with well-pronounced metachromasia (Fig. 2, left and centre). The hyaline-fibrinoid deposits took the form of focal subendothelial strips, pools or cores exhibiting various luminal and/or medial expansion. Small linear deposits were not usually seen in H & E-stained sections. Voluminous insudates often assumed a transmural character with typically humpy protrusions into the adventitia, the insudative "knobs" (Fig. 1, bottom). Even in

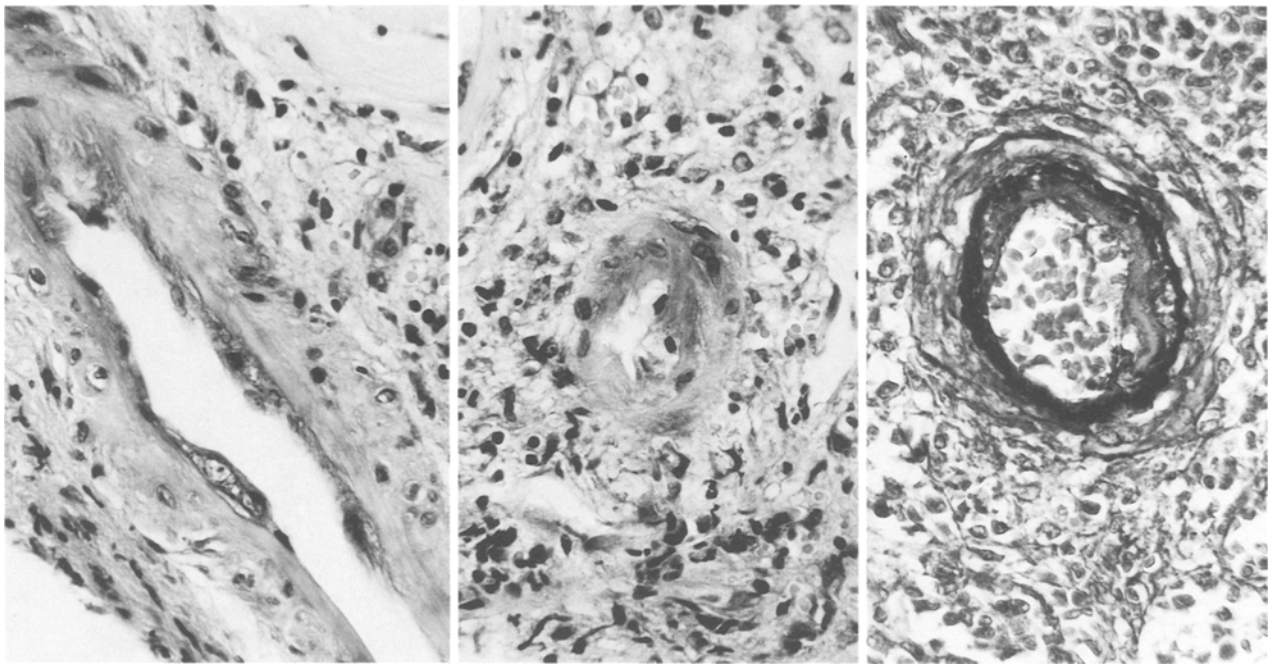


Fig. 2. *Left*: Oedematous intimal thickening with accumulation of metachromatic substances, end portion of an interlobular artery. Azur-eosin, ×420. *Centre*: Accumulation of metachromatic material in the wall of an afferent arteriole (*upper half*). Polymorphism and pyknosis of endothelial and smooth muscle cell nuclei, patency of residual lumen. Azur-eosin, ×420. *Right*: Small parietal microthrombus in an arteriole; upper and right segment, aldehyde fuchsin, ×420. These micrographs are from two of the three grafts excised at the beginning of our cyclosporin A (CsA) programme, when participation of CsA toxicity in graft loss was assumed

such massive insudates the slit-like remains of the lumen could usually be recognized. Thrombosis was rare and was seen almost exclusively in CsA-treated patients. It presented as mural strips and coats rather than as occlusive plugs (Fig. 2, right). The endothelial cells displayed focal pyknosis and nuclear polymorphism, sometimes with vacuolization; lipid-laden foam cells were not seen. Intimal proliferation typical of OA never occurred in IVA. The medial muscle cells displayed focal polymorphism and swelling but were flattened close to larger deposits. Segmental defects of media accompanied extensive insudates, chiefly as transmural knobs. No leucocytic invasion of the vascular wall was seen.

In immunofluorescence microscopy 23 biopsies contained cortical arterioles (15 in CsA- and 8 in AZA-based immunosuppression). C 3 was a constant finding in the form of small intramural granules and strips. In one-third of biopsies parietal layers and intimal pools of IgM were seen, whereas IgG or fibrin was exceptional and IgA did not appear. No difference in the extent and shape of fluorescent material was noted between the two groups of different immunosuppressive therapy.

In the CsA-treated patients (Table 1) transmural "knobs" were always considered to represent severe IVA and occurred in about 40% of this group. They were found 5 weeks to 24 months post Tx (mean 7.9 months). The metachromatic deposits appeared in both severe and moderate IVA 5 weeks – 24 months (mean 6.5 months) and 4 weeks – 14 months (mean 4.6 months) post Tx, respectively. The structures of microcoagulation were rather infrequent in severe IVA (1–5 months, mean 2.1 months post Tx) but exceptional in moderate IVA (after 6 months). The isometric tubular microvacuoles appeared in both severe and moderate IVA (as well as in IVA-free biopsies).

Table 1. Prevalence of characteristic microscopic structures of insudative vasculopathy (IVA) in patients treated with cyclosporin A (CsA)

	Severe IVA ($n=32$)	Moderate IVA ($n=30$)	P
Transmural insudates ("knobs")	12 (37.5%)	0	(<0.001)
Oedematous intimal thickening (metachromasia)	13 (40.6%)	7 (23.3%)	NS
Mural microthrombi	7 (21.9%)	1 (3.3%)	<0.05
Microvacuoles of proximal tubules	7 (21.9%) (5× well-marked)	8 (26.7%) (3× well-marked)	NS

In conventional treatment typical "knobs" were not encountered even in extensive stenosing insudates. Similarly oedematous intimal thickening and microthrombosis in arterioles were absent. Sporadic tubular microvacuoles suggested an early osmotic lesion (mannitol) or steatosis in graft glomerulonephritis (GN).

Electron microscopy in CsA-treated patients revealed graft arterioles in 22 biopsy samples (1985–1988) collected 3 weeks–34 months (mean 9 months) post Tx. Fourteen biopsies were performed within the 1st year.

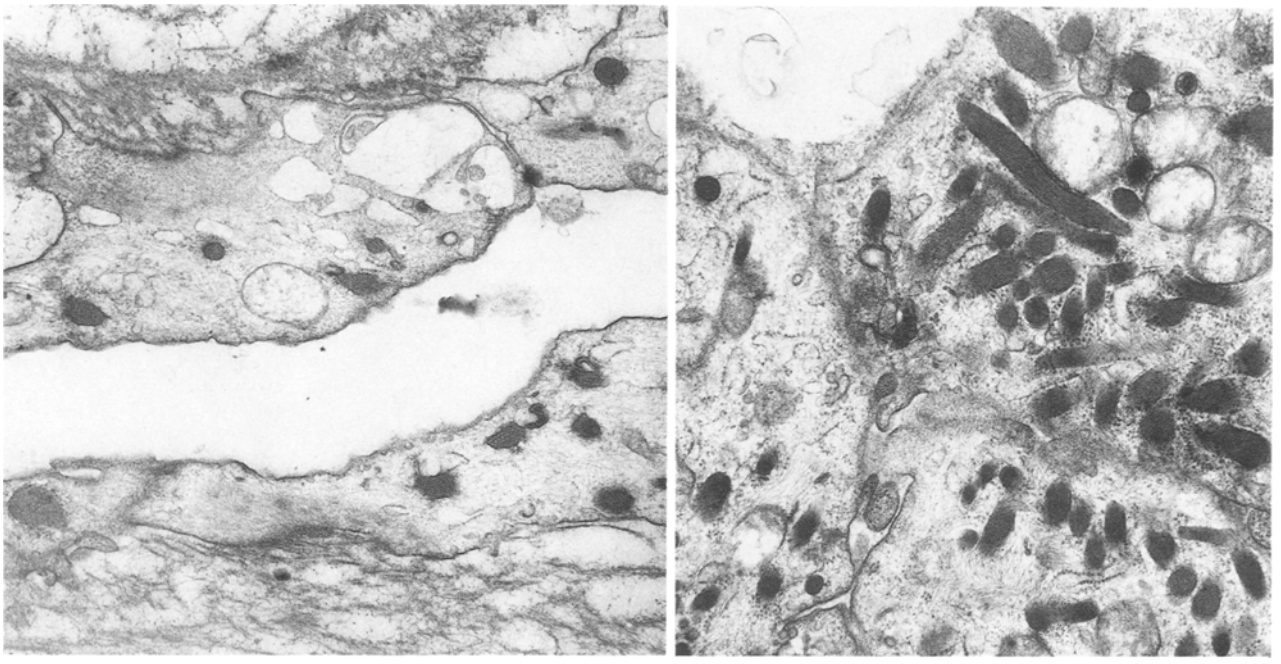


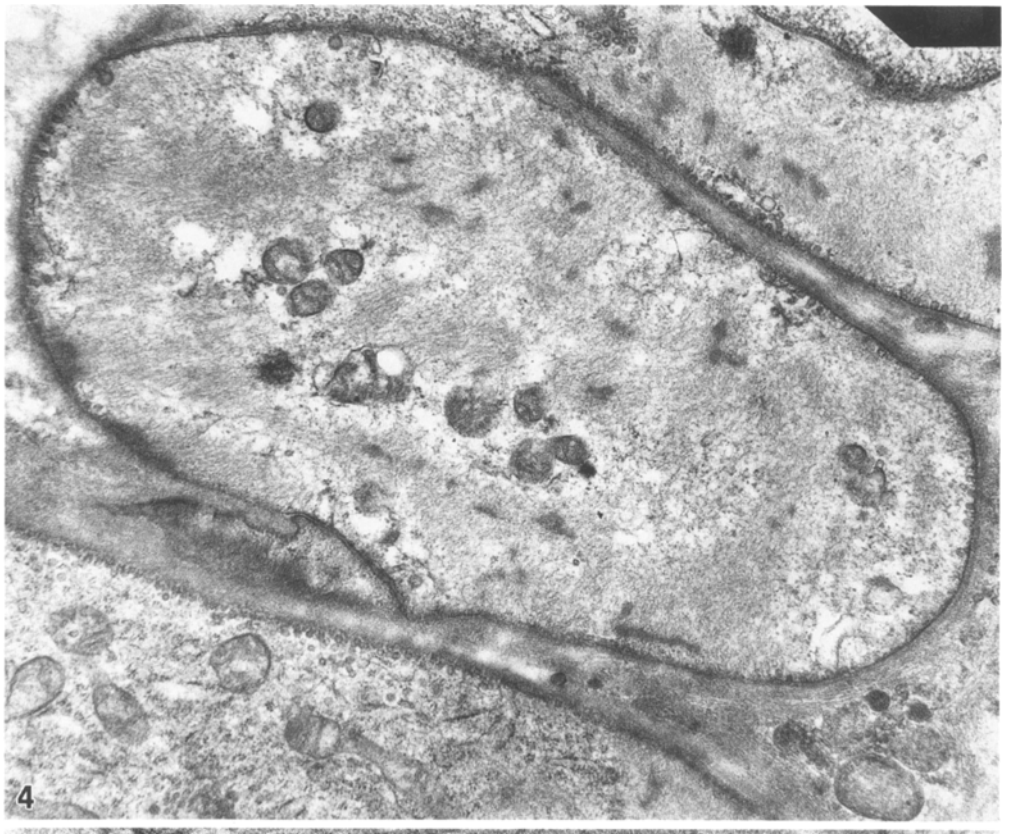
Fig. 3. Left: Endothelial swelling, vacuolization, and multiple dense cytoplasmic inclusions of an arteriole. ×25300. *Right*: Extreme increase in endothelial dense bodies. ×27500

Light microscopy revealed stenosing or obliterative IVA in 8 of these patients, 4 had minor insudates, and 10 biopsies were free of insudative lesion. Three of these latter cases showed oedematous intimal thickening. The endothelial cells displayed focal, sometimes stenosing cytoplasmic swelling with paucity of organelles (a similar oedema involved glomerular endothelial cells). Rare cells harboured lipid droplets or myelin figures. An increase in dense intracytoplasmic granules or rods (Fig. 3) with a simple limiting membrane, 100–200 nm in diameter was obvious in some cells. Some smooth muscle cells showed perinuclear increase of both smooth and ergastoplasmic vesicles and hypertrophic Golgi zones; myelin figures were exceptional. A prominent structure of some biopsies was the rarefaction of myofibrils with focal or confluent translucent "moth-eaten" defects, clumping, disappearance of fusiform densities and defects of the adjacent peripheral microvesicles (Figs. 4, 5). The extent of damage varied between circumscribed areas and total disarray of the cell contractile system. Other biopsies exhibited signs of regression or apoptosis of individual SMC with shrinkage, density of cytoplasmic matrix, vacuolization and extracellular accumulation of cell rests (Fig. 6). In the neighbourhood of large insudates signs of atrophy and compression prevailed: thin strands and ribbons of displaced cells were poor in organelles but expressed a regular net of myofibrils.

The insudates had a dense finely granular matrix, often with discrete translucent holes, slits and compartments. There was an inconstant admixture of membranovesicular fragments, mainly close to SMC. The large compact insudates tended to display a uniform homogeneous granularity and contained no typical fibrin or platelet structures. The insudative areas presented as strips and patches of varying extent in the subendothelial and intercellular medial spaces. The transmural "knobs" formed voluminous prominent granular masses expanding into the adventitia and displacing SMC and bundles of collagen (Fig. 7). The inner elastic membranes exhibited lamellar thickening of various degree with focal invagination and squeezing of SMC into the subendothelial area. The intimal oedema of light microscopy corresponded to large foci of translucent cavitation and honeycombing of the basement membrane matrix with a net of delicate non-periodic fibrils and minute, highly dense granules (Fig. 8).

In CsA-treated patients the endothelial oedema (5 cases) and hyperplasia of dense granules (6 cases) occurred, often together in the early post-Tx period (that is, up to 2 months) mainly in our first CsA patients (1985–1986). They did not correlate with the presence and extent of insudates. Similarly the defects of leiomyofibrils in 11 biopsies, (extensive in 7) represented an early lesion (3 weeks to 18 months, mean 5.4 months post Tx; 8 times in the first semester) and were also seen chiefly in the initial "high-dose" period of our CsA programme, until 1986. The insudates were seen by transmission electron microscopy (TEM) in 8 biopsies and were large or transmural in 6 samples. Unlike the abovementioned cellular lesions they often represented a late post-Tx finding (1.7–34 months, mean 13.9 months; in 6 patients after more than 6 months) and were found in the last 2 years (1987–1988). The prominent regression and shrinkage of SMC (8 cases) also prevailed in the biopsies performed after 1986.

In conventionally treated grafts arterioles were examined by TEM in 10 biopsies of the late post-Tx period (14–50 months, mean 31.6 months; 8 patients showed GN or focal and segmental glomerulosclerosis of graft). Arteriolosclerosis was seen in 3 samples by light



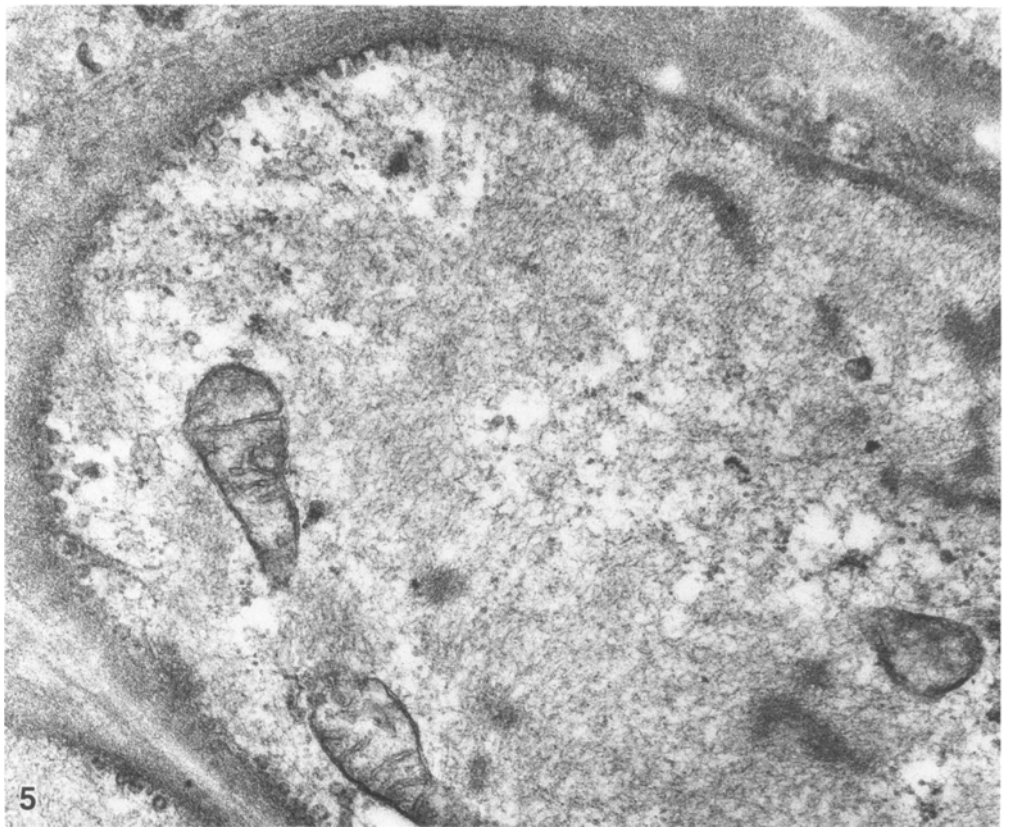
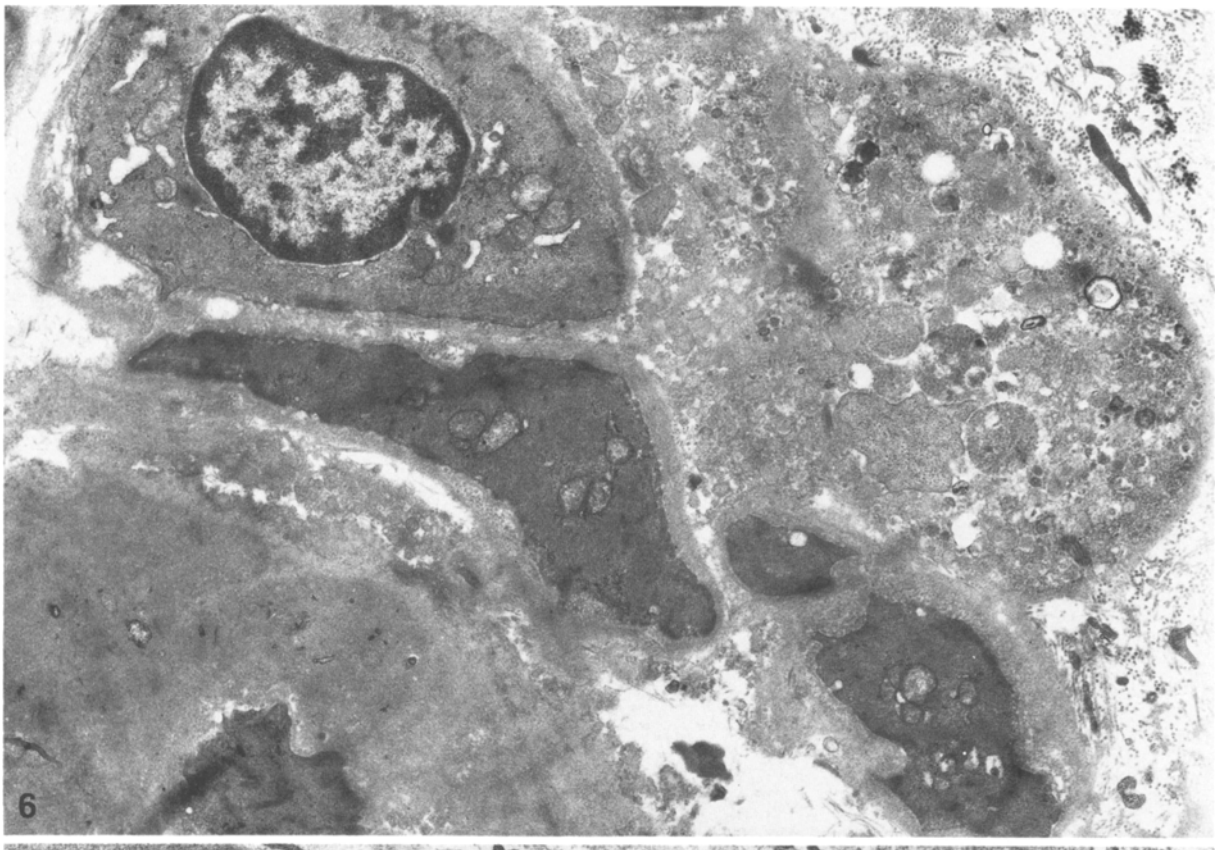


Fig. 4. Tunica media of an arteriole with irregular defects and gaps of contractile fibrils in smooth muscle cells. ×18700

Fig. 5. Detail of a smooth muscle cell to show the disarray and clumping of myofibrils. Slight oedema of mitochondrial matrix. ×46300



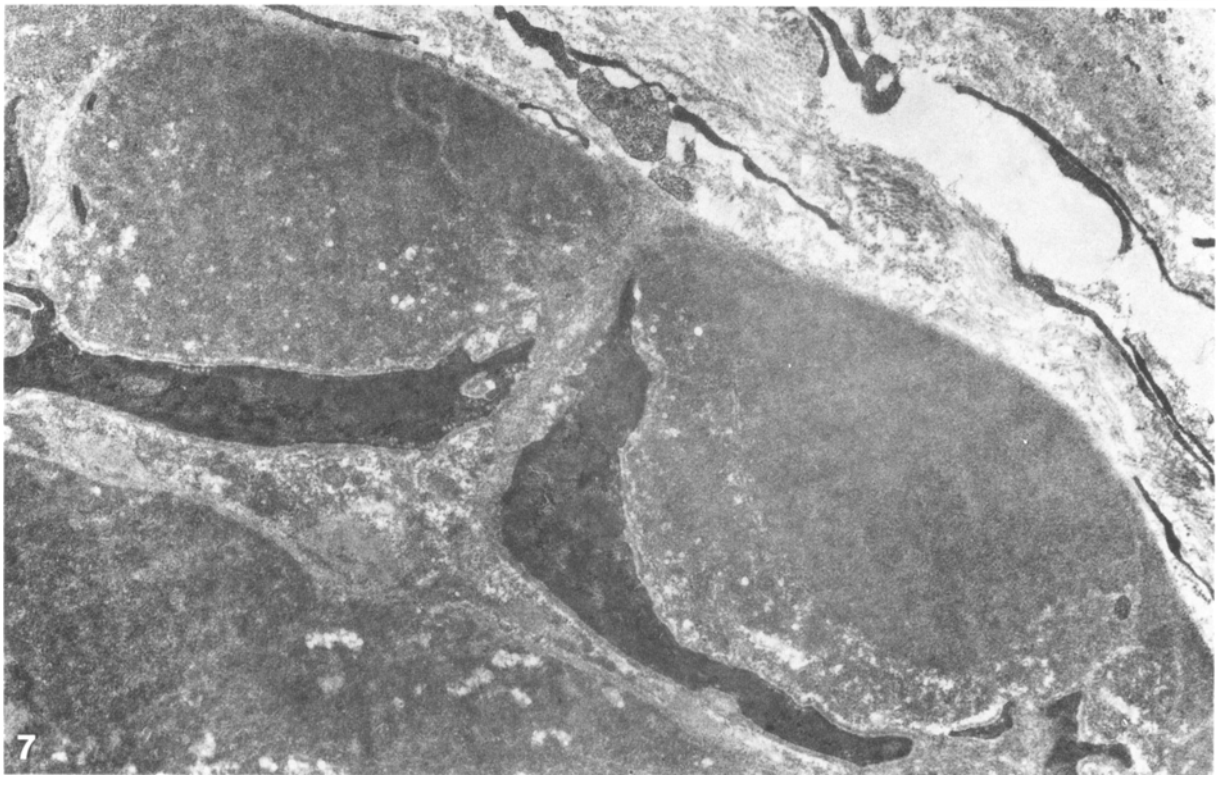


Fig. 6. Degenerative changes of arteriolar media in IVA. Focal shrinkage and density of smooth muscle cells (*centre* and *lower right*), vacuolization, and mural hyperchromatosis of nucleus (*top left*). No disarray of myofibrils (cf. Figs. 4 and 5). A voluminous accumulate of cell debris is seen at upper right; a granular subendothelial insudate is at lower left. ×11200

Fig. 7. Ultrastructure of a massive transmural insudate with two protruding "knobs" (adventitia is at *top right*). Note shrunken compressed muscle cells of media. *Bottom left*: Part of stenosing subendothelial insudate. ×10900

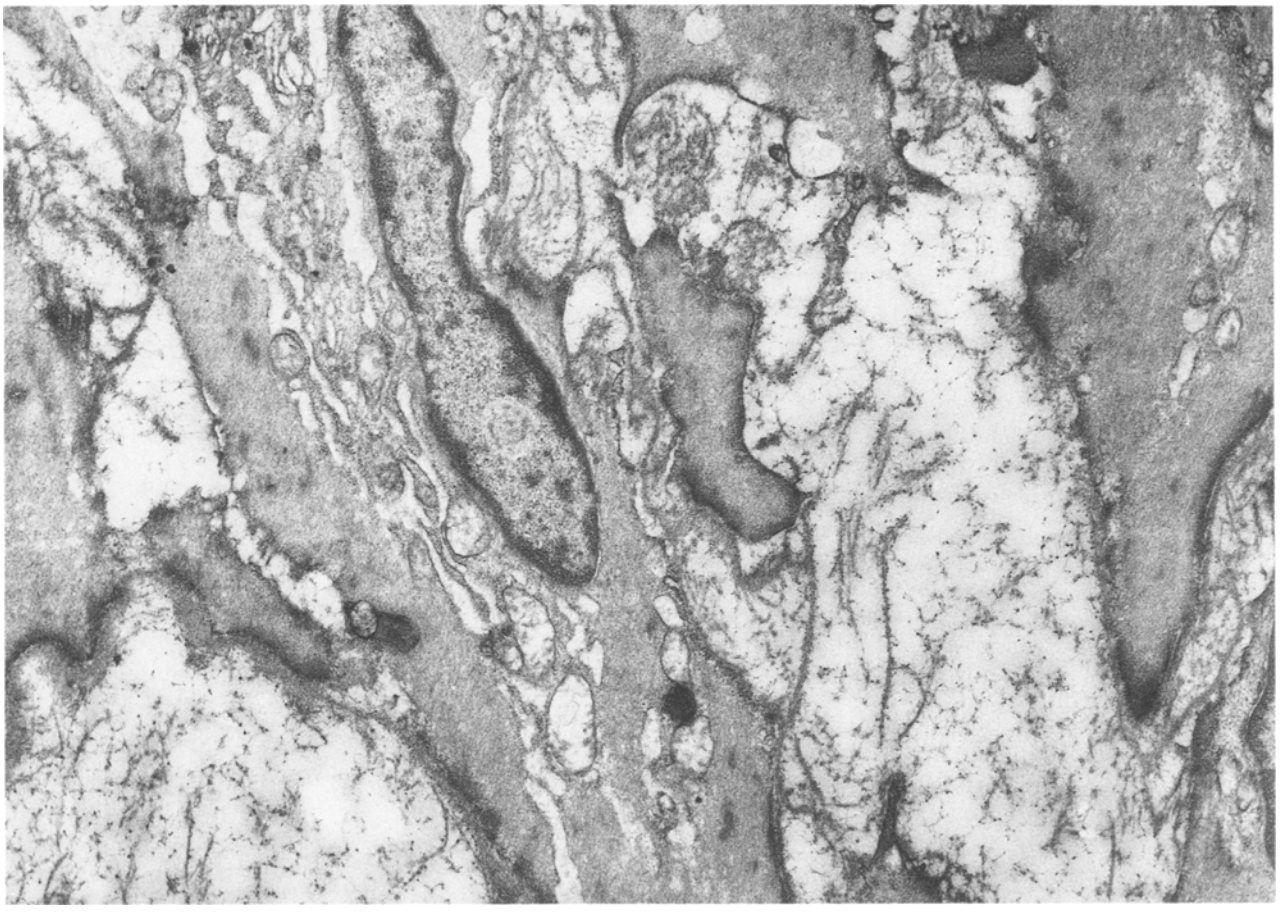


Fig. 8. Transmission electron microscopy of an area of arteriolar wall oedema with metachromasia (cf. Fig. 3, *centre*). Rarefaction of basement membrane matrix with fine non-periodic fibrils and minute dense granules. Hyperplasia of endoplasmic reticulum in a smooth muscle cell (*top* and *centre*); no disarray of myofibrils. ×15600

microscopy (28, 43, and 50 months post Tx). The ultrastructure did not show endothelial oedema or sloughing, and rare dense granules were found in only 2 samples. Large or transmural insudates ultrastructurally similar to those of CsA patients were found in 2 biopsies but did not exhibit typical "knobs". Small focal defects of leiomyofibrils appeared 4 times. The signs of muscle cell dystrophy were discrete or absent and the insudates caused thinning and displacement of cells without shrinkage or disruption.

Table 2 presents the results of semiquantitative scoring of JGL areas in IVA in 50 samples from CsA-treated patients. In biopsies with severe stenosing IVA negative or discrete JGL granulation was rare and massive granularity occurred more frequently. The post-Tx interval of massive hypergranularity was about one-half of that with moderate JGL granulation. The average age of donors was about 35 years, except the group of moderate IVA without hypergranularity (younger donors). In repeated biopsies of severe IVA (11 samples, after 0.75–8 months) the score did not change in 8 and decreased in 3 patients, all with persistent or progressive insudates. Two rebiopsies in moderate IVA (0.75 and 14 months later) displayed the same granularity as the first investigations.

Table 2. Juxtaglomerular (JGL) granulation in biopsies of CsA-treated patients with IVA

	JGL score	Severe IVA ($n=24$)	Moderate IVA ($n=26$)
Number of biopsies	0	2	10 $P<0.05$
	+	14	11 NS
	2+	8	5 NS
Post Tx interval (months)	0	5, 5	1.25–25 (mean 10.8)
	+	1–38 (mean 9.44)	0.1–18.5 (mean 7.7)
	2+	0.25–9 (mean 4.25)	0.75– 9 (mean 4.2)
Age of donor (years)	0	34, 53	16–48 (mean 29.7)
		13–61 (mean 36.1)	21–53 (mean 36.1)
	2+	17–48 (mean 35.4)	15–53 (mean 35.8)

Table 3 summarizes the annual numbers of Tx and graft biopsies, and the annual findings of moderate and severe IVA. The increase in IVA, especially of severe degree, has become inordinately high since 1984–1985 as compared to the slow increase in Tx activity and quantity of biopsies. We have also compared the frequency of biopsies in CsA- and AZA-treated patients. During 1979–1985 biopsies were obtained from 116 out of 162 conventionally treated patients with 2 years' or

Table 3. Annual prevalence of IVA

Year[a]	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988
All Tx/year	42	41	41	42	62	51	70	67	74	78
All biopsies/year	40	47	55	54	71	74	71	84	90	71
IVA in conventional therapy	5	5	6	6	13	11	8	11	6	2
IVA in CsA	0	0	0	0	0	2	11	23	30	18
All IVA – moderate	2	4	4	5	9	6	9	24	22	8
All IVA – severe	3	1	2	1	4	7	10	10	14	12

[a] Eleven Tx with conventional (azathioprine, AZA) therapy performed before 1979
Statistical significance (test of relative frequencies)
1979–1982 vs 1985–1988 $P<0.01$
1983–1984 vs 1986–1987 $P<0.01$
1979–1982 vs 1983–1984 NS
General linear trend 1979 through 1988 $P<0.001$
1985 through 1988 NS

Table 4. Age of graft (months) at the finding of IVA, prevalence of vascular rejection lesions and glomerulonephritis (GN)

A. Conventional immunosuppression	
1. Moderate IVA ($n=38$)	0.25–116 (mean 28.9) Rejection lesions: OA 5×, EA 20× Graft GN 12× (31.6%)
2. Severe IVA ($n=18$)	0.25–98 (mean 42.4) Rejection lesions: OA 0, EA 12× Graft GN 11× (61.1%)
B. CsA immunosuppression	
1. Moderate IVA ($n=30$)	0.25–25 (mean 6.6) Rejection lesions: OA 2×, EA 12× Graft GN 4× (13.3%)
2. Severe IVA ($n=32$)	0.3–37 (mean 6.5) Rejection lesions: OA 3×, EA 12× Graft GN 5× (16.1%)

EA, Rejection endarteritis; OA, obliterative arterio(lo)pathy
Statistical significance
A 1 vs A 2 NS A 1 vs B 1 $P<0.01$
A 1/A 2 vs B 1/B 2 $P<0.05$ (χ^2)
B 1 vs B 2 NS A 2 vs B 2 $P<0.01$

Table 5. Morphology of arterioles in earlier and subsequent biopsies[a]

A. AZA-based immunosuppression	
1. Moderate lesion	
previous samples ($n=8$)	IVA absent (8–101, mean 39.5 m)
subsequent samples ($n=8$)	7× persistence (1–35, mean 18.7 m) 1× slight progression (23 m)
2. Severe lesion	
previous samples ($n=4$)	2× IVA absent (6, 27 m) 2× moderate (23, 30 m)
subsequent samples ($n=5$)	4× persistence (1, 7, 11, 26 m) 1× partial regression (94 m)
B. CsA-based immunosuppression	
1. Moderate lesion	
previous samples ($n=2$)	IVA absent (12, 22 m)
subsequent samples ($n=3$)	2× persistence (1, 2 m) 1× progression (13 m)
2. Severe lesion	
previous samples ($n=7$)	1× IVA absent (20 m) 6× moderate-discrete (1–19, mean 5.8 m)
subsequent samples ($n=11$)	1× regression (1 m after conversion to AZA) 5× persistence (1, 1, 2, 7, 12 m) 5× progression (1, 1, 13, 24, 27 m)

[a] Months before or after the diagnostic biopsy

longer graft survival. In the CsA group, 63 out of 90 such patients underwent biopsy; the difference is not significant and it follows that in only 1 in 4 of our longer-surviving grafts biopsy was not needed.

The age of the donor was known in 112 Tx and averaged 34.74 and 35.58 years in the AZA and CsA groups, respectively (not significant). In the AZA group there was no donor age difference between the grafts with moderate and severe IVA (about 27% of donors were 30 years old or less) whereas in CsA severe IVA was found in older donors' kidneys (13–61 years, mean 37.84 years vs 15–53 years, mean 31.04 years in moderate IVA; $P<0.05$). The proportions of young donors also differed (16.12 vs 46.15% in severe and moderate IVA).

The post-Tx intervals at the biopsy finding of IVA and the frequency of concomitant vascular rejection changes and GN are given in Table 4. In cases with AZA treatment IVA was a very late post-Tx finding, whereas with CsA both moderate and severe IVA appeared within the first semester; the differences are significant for both the two subgroups.

Twenty-one samples were examined that had been obtained before the first diagnostic biopsy, which showed moderate or severe IVA. Twenty-seven rebiopsies were performed (Table 5). In AZA grafts the early biopsies always displayed normal arteries or small focal changes, in subsequent severe IVA. In rebiopsy the insudates persisted or progressed slightly. In CsA treatment the early samples showed no, or few insudates in the

Table 6. Pair analysis of cumulative graft survival (CsA therapy)

Post Tx period (months)	IVA absent ($n=28$)	IVA present ($n=28$; 5 moderate, 23 severe)
6	0.71 (28)	0.79 (28)
12	0.56 (19)	0.68 (28)
24	0.28 (14)	0.54 (19)
36	0.28 (8)	0.38 (18)

Wilcoxon (Breslow) $P=0.264$
Mantel-Cox $P=0.158$

severe IVA. A marked progression appeared in one-half of repeated biopsies; partial regression 4 weeks post-conversion was a single exception.

In clinical signs and outcome there was no apparent difference between the IVA and IVA-free grafts in pre-biopsy serum creatinine (S_{cr}) levels or urinary findings, except in graft GN. The mean CsA blood levels reached 387.9 ± 239.9 and 381.8 ± 277.9 ng/ml in moderate and severe IVA, respectively (not significant; calculated from 388 and 390 pre-biopsy readings). In the AZA group pre-biopsy hypertension ($\geqq 95$ mmHg diastolic) was noted in 62.5% and 53.8% of patients with moderate and severe IVA, respectively; for the CsA patients these proportions were 36.4% and 50.0%. The correlation between blood pressure and JGL granularity did not reach statistical significance. No significant difference in cumulative graft survival was found between moderate and severe IVA in either AZA- or CsA-treated patients; the same applied in 28 selected pairs comprising one IVA and one non-IVA subject each (Table 6). After the biopsy 15 out of 32 patients with severe IVA were converted to AZA, whereupon S_{cr} decreased in 5, did not change in 2 and increased in 8 cases. In 5 of these signs of active rejection were seen: the subsequent anti-rejection therapy led to improvement or stabilization in 3 of them. Before 1989, 54 grafts had lost their function. They were 18 of 38 of those with moderate and 15 of 18 with severe IVA in AZA-treated subjects, and 8 of 30 and 13 of 32 with CsA, respectively.

In excised grafts from the CsA group ($n=20$) with severe IVA in previous biopsies (9 cases), the main finding was vascular rejection damage in 7 cases, mainly in the form of EA. Only in 2 cases was the graft loss ascribed to the combination of IVA and microthrombosis; 1 of these 2, however, also displayed OA. In the moderate IVA-CsA subgroup 9 losses among 11 grafts were also related to a vascular rejection lesion and 1 graft showed microthrombosis. Two grafts succumbed to early surgical accidents. In the conventional group ($n=22$; 18 histological findings available) rejection vascular damage predominated in 12 cases; type I membranoproliferative GN appeared in 3, necrosis or infarction in 2, and severe non-rejection ischaemia causing acute tubular necrosis (ATN) was seen in 1 kidney.

During 1969–1978, 37 cadaverous kidneys were conserved by single perfusion and cold storage but were not transplanted for various reasons. In 21 of those no fibrinoid or hyaline arteriolar changes were noted. The donor age, known in 18 cases, was 3–48 years in 14 males (mean 24.0; after the exclusion of the youngest child 25.6 years) and 32–43 years (mean 38.2) in 4 females. Non-stenosing insudates or hyaline patches were occasionally recorded in 15 kidneys. The age range ($n=11$) was 32–53 years in 8 males (mean 41.3) and 42–53 years in 3 females (mean 49.7). In 1 case prominent stenosing IVA was seen in a 49-year-old man who died of cerebral infarction, thrombosis of right carotid artery, and general atherosclerosis including renal artery involvement. Arteriosclerosis of the fibroelastic type was present in 22 kidneys and in 1 it assumed a stenosing character.

Discussion

During the period of conventional immunosuppression, until 1983, our attention was focused on the rejection vascular changes, while IVA represented a rare finding of borderline significance. A turning point was marked after the introduction of CsA in all our Tx since September, 1985. The dosage of CsA and acceptable blood levels were repeatedly reduced thereafter, but this failed to effect a significant change in prevalence and morphology of IVA until 1989. Increases in the "ateriolosclerosis-like" graft lesion seen in between 15% and 60% of patients have been reported by other centres (Porter and Bennett 1986; Mihatsch et al. 1988; Thiel et al. 1988; Wilczek et al. 1988). The multicentre study (Bergstrand et al. 1985) documented IVA in 33% of biopsies but, somewhat surprisingly, also in 25% of AZA-treated grafts. This contrasts with the total absence of IVA in conventional treatment and 62% or 32% in CsA reported by others (Antonovych et al. 1988; Larsen et al. 1988). Some authors have reported a very low proportion of IVA in CsA treatment (Nizze and Brockmöller 1987) or a discrepancy between the frequent "toxic" striped fibrosis of the interstitium and the scarcity of IVA (Klintmalm et al. 1984). Such a disagreement may depend on different post-Tx intervals at biopsy as well as differing patient groups, on differences in semiquantitative assessment, and the difficult differentiation from or combination with rejection changes in some biopsies. In addition, the indication for biopsy is given by various changes, many of which will not depend on CsA toxicity or IVA itself. In late stages the insudates lose their fibrinoid character and are less readily discovered: Mihatsch et al. (1986) recommend a thorough search for IVA in all previous biopsies if the striped pattern of interstitial fibrosis appears in the late post-Tx stage.

About three-quarters of all our grafts, both on AZA and CsA therapy, have been examined by biopsy. If this group can be taken to be representative of the whole of our patients, then we find IVA in 14.7% of CsA- and in 5.1 of AZA-treated Tx ($P<0.01$). We cannot exclude the possibility, however, that some of our non-biopsied patients also had IVA of moderate degree at least; the real prevalence would then be somewhat higher in both AZA and CsA subgroups. Finally, CsA permits a longer graft survival, which theoretically yields more

time for the development of insudates: however, given the early onset and more rapid development of IVA in our CsA patients, this does not seem to be significant.

Our comparative group of 37 non-used cadaver kidneys (Rossmann et al. 1980) displayed normal arteries or a discrete to moderate arterio(lo)sclerosis with two exceptions. These two organs with a stenosing vascular lesion were collected in the very first years of our Tx programme and certainly would be refused after 1975 with the revised criteria for organ sampling. It follows that if basic rules of kidney donation are respected the risk of transfer of a significant insudative lesion is minimal and that IVA is a disease of the graft and not of the donor. Some centres refer to worse results for Tx where the donors' age exceeds 40 (Leunissen et al. 1988), but the age of 60 which represents our limit, or even 65 years has been widely accepted (O'Connor et al. 1988; Terasaki et al. 1989). In our CsA group patients with severe IVA had received the older donors' kidneys: however, the age difference is of borderline significance and in view of the shortage of transplantable organs no further restrictions in donor choice are needed.

However, the post-Tx development and dynamics of IVA largely depend on the mode of immunosuppression. In conventional treatment IVA is a slow and torpid process known since the very beginning of the Tx area as a stenosing "fibrinoid necrosis of afferent vessels" with local deposition of IgM and fibrin (Starzl et al. 1964; Busch et al. 1971). In our AZA group IVA was a late finding with a striking tendency to morphological arrest without symptoms or signs which allowed clinical recognition. As most of our biopsies are taken within the first post-Tx months (rejection episodes, OA etc.), it is highly improbable that a symptomless insudative process might develop without being noticed in the early post-Tx period or fail to be diagnosed later. In CsA-based therapy, however, IVA represented a far earlier finding without an apparent interval between moderate and severe insudates. Severe IVA can be found, albeit rarely, as soon as 2 months post Tx (Mihatsch et al. 1986). The main sign of CsA-associated IVA is believed to be a slow creeping functional deterioration, usually in the second post-Tx semester or later (Thiel et al. 1983; Sommer et al. 1986). This trend was not very clearly expressed in our patients and the 3-year graft survival in severe IVA matched that in moderate IVA and even in IVA-free grafts. The degree of IVA correlated neither with the presence of isometric "toxic" tubular vacuoles (Mihatsch et al. 1983), a rare finding in our biopsies since 1986, nor with the average or median CsA blood levels.

IVA is an irreversible lesion which also develops and progresses in the autologous kidneys of CsA-treated patients (Berg et al. 1986; Nizze et al. 1988); significant damage of the renal vasculature is to be expected after 6–12 months of uninterrupted CsA therapy (Myers 1986). The frequently seen post-conversion functional improvement (Thiel et al. 1983) does not correspond to the restitution of vascular morphology (Sibley et al. 1987). However, the consequences of IVA are less serious than might appear from morphology: in our 42 excised grafts late rejection changes were by far the most salient finding. In only 2 cases was IVA considered to be the main cause of graft loss, while another graft had microthrombosis, and 2 of these 3 grafts also showed early vascular rejection damage. Thus the presumably CsA-dependent lesions have rarely, if ever, caused graftectomy in our centre.

IVA is most likely to be a lesion unrelated to rejection. Its morphology differs from OA and EA and its exclusive target is the arteriolar wall; the rejection process prefers small and larger muscular arteries, respectively. Furthermore, OA has been greatly reduced by CsA treatment (Ballardie et al. 1986). In our centre its prevalence fell from 23% to about 8% of patients ($P < 0.001$; Rossmann et al. 1986), but as has been shown, the opposite happened with IVA. The non-rejection origin of IVA is also substantiated by its development in autologous kidneys of CsA-treated patients (Myers et al. 1984; Nizze et al. 1988). Nevertheless the morphological distinction may be difficult; the co-existence of two vascular lesions is possible, and the insudative process itself may be modified by the rejection (Mihatsch et al. 1986).

We noted a clearcut relationship of some ultrastructural details of IVA to the post-Tx interval and to the length of our experience with CsA. The endothelial swelling was a typically early lesion seen at the beginning of our CsA programme with higher drug doses and levels. It also involved glomerular capillaries, sometimes resembling pre-eclamptic nephropathy (Mautner et al. 1962; Aber 1978) but was not accompanied by platelet aggregation or focal cell sloughing as is seen in subacute rejection (Rossmann et al. 1975). Endothelial swelling can also be elicited by an experimental CsA load in rats (Benigni et al. 1988). Like oedema, the hypergranularity of endothelial cells was seen in the early post-Tx period at the beginning of CsA treatment programme and has also been observed by others (Antonovych et al. 1988). The contents and role of these granules have not been defined as yet, but they resemble Weibel-Palade bodies (Casley-Smith 1980), the biological reservoir of factor VIII (Warhoe and Sweet 1984). They accumulate in endothelial cell culture after the supplement of CsA to the medium (Ryffel et al. 1988). Similar hyperplasia was noted by us in the subendoicardial arterioles of CsA-treated cardiac allografts (1986, unpublished data). Hypergranulation was also seen in various experimental lesions such as renovascular hypertension, irradiation or endotoxin load (Detre et al. 1986; Reidy et al. 1989). Another prominent early lesion was cytoplasmic disarray of SMC in the form of focally clumped and torn-off myofibrils. This also occurred during the first 2 years of our CsA experience and was discrete or absent in AZA treatment. A similar lesion was noted in CsA-treated renal allografts by others (Yamaguchi et al. 1989) and appeared in most of our above-mentioned cardiac allografts. Nevertheless the disorganization of myofibrils may be non-specific and has been recorded in the experimental hypertension of rats (Aikawa et al. 1970) or in protracted cerebral vasospasm of primates (Alkane 1974).

The insudates were found distinctly later post-Tx than the cellular lesions and appeared later in our CsA programme. They evidently have not been eradicated

or mitigated by repeated changes in CsA medication. In about one-half of severe IVA some insudates took the form of bulky transmural bulging humps, here referred to as knobs and by others as clover-leaf or necklace insudates (Mihatsch et al. 1988) or nodular hyalinosis (Yamaguchi et al. 1989). These knobs seem to be a distinct though not pathognomic feature of CsA-associated IVA: we failed to find such typical nodules in the AZA-treated allografts or in arteriolosclerosis of autologous kidneys. In CsA treatment the insudates usually left residual luminal slits devoid of intimal proliferation; they were not surrounded by foam cells and did not initiate leukostasis or cell invasion. These details are useful for differential diagnosis of OA, EA and vasculitis (Rossmann and Jirka 1979). By TEM the insudates had – in both AZA and CsA – two characteristic components: (i) a dense granular precipitate and (ii) fragments of membranes and organelles. In the voluminous insudates including knobs, the background granular substance was more evident than the cell debris. Like the insudates, the dystrophy and disruption of SMC was a later post-Tx finding, seen throughout our CsA therapeutic programme. As stated by Mihatsch et al. (1988) the damage to SMC is far more vigorously expressed with CsA than in non-CsA arteriolosclerosis. This conclusion is backed up by our results, although our biopsies in the IVA-AZA group, obtained after longer post-Tx intervals and partly under different conditions, are not optimal comparative material and the recognition of SMC dystrophy by light microscopy is doubtful (Myers et al. 1988). By TEM our samples displayed cytoplasmic condensation and apoptotic shrinkage not usually accompanied by hyperplastic lysosomes, myelin figures or total cell disruption. The damage to SMC was not proportional to the insudation: large insudates or knobs were rimmed by slim compressed cytoplasmic strands devoid of shrinkage or torn-off myofibrils in most localities. The intimal oedema is morphologically distinct from the insudates (Mihatsch et al. 1986) and is best visualized by metachromatic staining. In our samples, its presence and extent did not correspond to that of insudates, and by TEM its appearance also differs from the insudates: focal honeycombed swelling of the basement membrane-like substance points to a degradation of extracellular matrix rather than to the prevailing insudative process. Pools of similar metachromatic material are frequent in EA but involve arteries of larger calibre.

The effect of CsA on renal function and intrarenal circulation has been documented extensively in both clinical and experimental conditions. The basic derangement is a protracted preglomerular vasoconstriction with a striking increase in peripheral resistance (Murray et al. 1985; Myers et al. 1988; Curtis and Laskow 1988) and our ultrastructural findings are compatible with prolonged vascoconstriction causing degenerative changes in SMC as the most important feature of IVA (Nizze et al. 1988). Morphology of IVA also points to an impairment of the ultrafiltration barrier, insudation, and eventual scarring of the arterial wall. A direct toxic effect of CsA on SMC is questionable: local accumulation of CsA has been shown neither in experimental loading (Ryffel et al. 1986) nor in human graft vessels (Kolbeck et al. 1987). A structural change pointing to protracted vasoconstriction is the frequent though not consistent JGL hypergranulation. In our biopsies clusters of coarse granules expanding into the adjacent SMC of vas afferens were frequent though not constant in severe CsA-IVA. The donor's age was of no apparent importance. A prominent CsA-associated JGL hyperplasia has also been observed in autologous kidneys (Myers et al. 1988). CsA promotes prorenin secretion in both explanted glomeruli (Kurtz et al. 1988) and in clinical conditions (Curtis and Laskow 1988) but conversion to the active renin is limited and its levels remain chronically low (Myers et al. 1984).

Microthrombi in arterioles and glomeruli were an infrequent finding in our biopsies, primarily in severe CsA-IVA. They occurred quite exceptionally in our AZA-treated patients, for example in activated rejection; here, however, peritubular capillaries were their main site. CsA has been shown to stimulate the in vitro production of coagulation factors in both endothelial cells and monocytes (Zoja et al. 1986; Petric et al. 1988) and to induce an in vivo increase in factor VIII (Brown et al. 1986). Intra-graft microcoagulation is a potentially dangerous complication of CsA therapy (Neild et al. 1985; Wolfe et al. 1986, Landmann et al. 1987) and may even assume a systemic character (Sommer et al. 1986). Gross thrombosis of main graft arteries has also been reported to occur more frequently with CsA treatment (Merion et al. 1984; Frödin et al. 1987; our unpublished data), but it is a complication of the first post-Tx days or weeks, lacking an apparent connection with IVA.

CsA has brought a significant therapeutic benefit but has also presented new problems in Tx research and routine. While the CsA tubulopathy and "toxic crises" have been largely mastered by adjustments in therapy, the vasospastic and insudative arteriolar lesion has persisted. Despite its striking and sometimes alarming morphology, the impact of IVA on the post-Tx course has proven less severe than appeared to be likely and loss of our grafts could rarely if ever be ascribed to CsA-associated pathology. However, these conclusions remain to be confirmed by revision of our results after a longer post-Tx interval.

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Note added in proof: In 1989 79 Tx were performed in our Centre and a total of 95 graft biopsies was obtained. IVA was found in 41 samples (34 findings in CsA-treated patients). In 25 casas IVA was of moderate and in 16 of severe degree. It follows that the prevalence of insudative arteriolar lesion did not decline in spite of repeated restrictions in CsA dosage (recommended blood levels 190–380 ng/ml in 1989). The difference of relative frequencies and the general prevalence trends shown in Table 3 are also valid for 1989.