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ORIGINAL ARTICLE

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The involvement of activated T cells and growth-factor production in the early and late phase of chronic kidney allograft nephropathy in rats

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Abstract T cells are thought to play a regulatory role in chronic allograft nephropathy (CAN). Thus, we investigated whether lymphocyte inhibition influences CAN. Fisher rat (F-344) kidneys were transplanted orthotopically into Lewis rats. Animals received cyclosporin A (1.5 mg/kg per day, s.c.) for 10 days and were treated daily with either cyclosporin A (1.5 mg/kg), tacrolimus (0.16 mg/kg), or a vehicle thereafter ($n = 15$ per group). Kidneys were harvested at 16 or 24 weeks. Interleukin-2 (IL-2) and interleukin-2 receptor β (IL-2Rβ) mRNA synthesis were intense at 16 weeks and decreased thereafter. Unsurprisingly, both cyclosporin A and tacrolimus significantly inhibited IL-2 and IL-2Rβ at both time points. Proteinuria increased more rapidly in controls than in treated animals. Morphologically, over 40% of glomeruli were sclerosed by 16 weeks in controls, and ED-1+ macrophages and CD5+ T cells infiltrated the graft. IL-2 mRNA synthesis paralleled the number of infiltrating cells. Inhibition of T-cell proliferation significantly reduced glomerulosclerosis and leukocyte infiltration at both time points. Transforming growth factor (TGF)-β_1 and platelet-derived growth factor (PDGF) synthesis were highly upregulated in controls at 16 weeks, the time of peak infiltration. At 24 weeks, as cellular infiltration was replaced by scar formation, TGF-β_1 mRNA returned to normal, while PDGF did not. Inhibition of T cells prevented the upregulation of TGF-β_1 at both time points; however, PDGF was suppressed only at week 16. These results indicate a beneficial effect of continuous suppression of T cells in CAN. T cells are probably more important in the early, inflammatory phase.

Keywords Chronic allograft nephropathy · T cells · Interleukin-2 · Transforming growth factor-β · Platelet-derived growth factor

Abbreviations *ED-1* monoclonal antibody against rat CD68 equivalent tissue-macrophage marker · *ICAM-1* (*CD54*) intercellular adhesion molecule 1 · *IL-2Rβ* (*CD122*) 70 kDa beta protein (p70) of the interleukin-2 receptor · *LFA-1* (*CD11a*) alpha chain of the leukocyte function associated antigen 1 · *OX-19* monoclonal antibody directed to rat CD5+ equivalent pan-T-cell marker · *TGF-β_1* isoform 1 of transforming growth factor beta · *VCAM-1* (*CD106*) vascular cell adhesion molecule 1 · *VLA-4* (*CD49d*) alpha integrin of very late activation protein 4

Introduction

Results of clinical transplantation have been markedly improved by the development of new immunosuppressive drugs. Despite these improvements, the rate of attrition over the long term remained constant [27, 45, 46]. The underlying cause is thought to be the ill-defined process of chronic allograft nephropathy (CAN). CAN has been hypothesized to be caused by cell-mediated permanently ongoing rejection of the present alloantigen [1, 43], recurrent intermittent acute rejection episodes [28, 36, 46], or alloantigen-independent processes that activate graft endothelial cells and induce an unspecific inflammatory response [21, 46]. The extent to which these mechanisms participate in the process of CAN is not yet understood.

Kidney grafts with CAN are infiltrated with mononuclear cells, including T cells. Interleukin-2 (IL-2), originally called T-cell growth factor [56], is responsible for the proliferation of T cells upon activation [1]. IL-2 and its receptor (IL-2R) are upregulated during the process of T-cell activation [41]. As IL-2- and IL-2R-positive lymphocytes have constantly been detected in chronically rejecting kidneys [18, 38], they may determine the pace of CAN [1, 10, 43], stressing the importance of alloantigen-dependent events. A number of recent studies deals with the role of T cells in CAN. Oligoclonal T cells [55] and T cells with antidonor specificity [25] were demonstrated in CAN of human heart allografts, indicating antigen-driven proliferation and clonal expansion of T cells. Furthermore, Kirk et al. have demonstrated that the number of lymphocytes infiltrating human kidney allografts and T-cell receptor turnover correlated with proteinuria, fibrosis, and late functional deterioration. It has also been described that CD8+ T cells induce apoptosis during chronic rejection [42, 52]. All these observations stress the importance of activated T cells in the process of CAN.

The development of interstitial fibrosis in CAN has been attributed to local transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF) production [46, 54]. In clinical biopsies of chronically rejecting kidneys, the TGF-β_1 isoform predominated [53]. As it has been well described that both cyclosporin A and tacrolimus induce TGF-β_1 in vitro and in vivo, it was hypothesized that continuous treatment with these drugs might be harmful over the long term. To test whether continuous immunosuppressive treatment is beneficial or harmful over the long term, we studied how inhibition of T cells by either cyclosporin A or tacrolimus interfered with the pace of kidney CAN in rats. We put a particular emphasis on their effects upon PDGF and TGF-β.

As re-transplantation experiments have shown earlier, CAN is reversible if re-transplantation is performed early after transplantation (within 8 weeks in the Fisher-to-Lewis rat kidney allograft model), but irreversible if performed later [51]. Thus, we hypothesized that at different times after transplantation, different mechanisms might operate during the process of CAN.

Materials and methods

Experimental animals

Naive male inbred Lewis (LEW) and Fisher (F-344) rats weighing 180–250 g were used throughout the experiment. All animals were obtained from Charles River, Munich, Germany, housed under standard conditions, and fed rat chow and water ad libitum. All animal experiments were carried out according to the principles of laboratory animal care [16], and the experimental protocol was reviewed and approved by a government animal care and research committee of Germany.

Renal transplantation

Fisher rats served as donors and Lewis rats as recipients. Transplantation was performed as previously described [21]. Briefly, the left donor kidney was perfused with Ringer's lactate (4 °C), removed, and positioned orthotopically into the recipient, whose renal vessels had been isolated and clamped and from whom the native kidney had been removed. End-to-end anastomosis of renal artery, vein, and ureter was performed, using 10-0 Prolene sutures. Total graft ischemia was less than 30 min. To prevent infectious complications during the perioperative phase, we administered 20 mg/kg ceftriaxone (Rocephin) daily during the first 10 postoperative days, at which time the right native kidney was removed.

Experimental design

Rats received 1.5 mg/kg b.w. cyclosporin A (Calbiochem Biochemicals, Bad Soden, Germany) for the first 10 postoperative days to prevent an initial episode of acute rejection. After day 10, animals were divided into three treatment groups ($n = 15$ per group) and received either 1.5 mg/kg b.w. cyclosporin A, 0.16 mg/kg b.w. tacrolimus (Fujisawa, Japan) [28], or a vehicle, on a daily basis. Both immunosuppressants were suspended in Cremophor-ethanol, according to the manufacturer's instructions and administered subcutaneously.

Drug dosages were determined in a pilot study. Transplanted rats chronically receiving 3.2 mg/kg/day cyclosporin A or 0.32 mg/kg/day tacrolimus for 2 months suffered from toxic side effects such as continuous loss of body weight and diarrhea. In the present study the highest dosages – without side effects – were applied. To exclude the influence of body weight on the extent of proteinuria, we matched body weight at the time of operation (260 ± 15 g). Weight differences between the groups did not reach statistical significance throughout the experiment. Complications of grafting (hydronephrosis, stones) were observed in two controls and in one tacrolimus-treated recipient. Those animals were excluded from further analysis. After 16 or 24 weeks, rats were anesthetized with diethylether, and the intra-aortic blood pressure was measured with a DPT 3003-S/3 cc arterial transducer (Peter von Berg Medizintechnik, Germany). Rats were bled thereafter, and the transplanted kidney was removed. Samples were snap-frozen in liquid nitrogen for immunohistological staining and for polymerase chain reaction (PCR) analysis, or fixed in buffered formalin (4%) for light microscopy.

Routine chemistry

For protein analysis, 24-h urine samples were collected every 4 weeks. Urinary protein and creatinine excretions as well as serum creatinine concentrations were determined by standard methods. The creatinine clearance was calculated at the end of the study.

Antibodies

Monoclonal antibodies against CD5+ rat T cells (OX-19), macrophages (ED-1), ICAM-1 (CD54), VCAM-1 (CD106), VLA-4α (CD49d), and LFA-1α (CD11a) were purchased from Serotec Camon Labor Service, Germany.

Histology and immunohistology

For histology, kidney tissues fixed in 4% buffered formalin were embedded in paraffin and stained with hematoxylin/eosin and periodic acid-Schiff so that we could assess the extent of glomerulosclerosis. Glomerulosclerosis was defined as a collapse of the glomerular capillaries, adhesion of the obsolescent segment of Bowman's capsule and the entrapment of hyaline [48]. Slides were scored in a blinded fashion, and the number of glomeruli with sclerotic lesions was expressed as percentage of the total number of glomeruli counted. A minimum of 200 glomeruli per kidney was evaluated.

For immunohistology, cryostat sections (4 μm) were fixed in acetone and stained individually with the alkaline-phosphatase-anti-alkaline-phosphatase (APAAP) technique as previously described [22] and counterstained with Mayer's hemalum (Merck, Darmstadt, Germany). Cells staining positive were counted on an ocular grid and expressed as cells per field of view (cells/fv). At least 20 field-of-view sections per specimen were counted at 400×magnification. The intensity of tissue staining (ICAM-1, VCAM-1) was evaluated in a blinded manner on a scale from 1 to 4, with 1 indicating minimal and 4 intense staining.

Reverse transcriptase polymerase chain reaction

We prepared RNA samples derived from frozen kidneys by using a guanidine isothiocyanate/phenol/chloroform isolation method (RNeasy, Total RNA Isolations Kit, Qiagen, Germany) [54]. For first-strand cDNA synthesis, 1 μg of total RNA was used with oligo[dT]$_{12-18}$ as primer under supplier-recommended conditions (GIBCO/BRL). Specific cDNA amplification has been described previously [9]. Briefly, specific cDNA products complementary to mRNA sequences of rat TGF-β_1, IL-2, IL-2Rβ (p70) (CD122), and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (Euro Gentec, Belgium) were amplified using the polymerase chain reaction (PCR) [38]. A master mix containing reaction buffer, magnesium chloride, deoxynucleoside triphosphate, and Taq DNA polymerase (DIANOVA) was set up. Specific primers and sample cDNA were added to each master mix. A Perkin-Elmer Thermal Cycler (Model 2400) was used for amplification. The amplified PCR product was electrophoresed on a 1.5% agarose gel and stained with ethidium bromide, and gene fragments were visualized by UV light. Quantity of PDGF-AB, TGF-β_1, IL-2 and IL-2R cDNA was estimated by densitometric comparison with GAPDH (internal control) from the same sample after the positive image of the gel had been digitized for computerized densitometry.

Statistical analysis

Data are presented as mean ± standard error of mean (SEM). Differences between the three groups were assessed by Fisher's least-square distance test [19], and probability values between treated groups and control group or between the two treated groups were calculated by Student's *t*-test as appropriate. Non-metric parameters, such as the intensity of staining for adhesion molecules, were evaluated by Fisher's exact test for ordinal data [9]. A P value of less than 0.05 was considered significant.

Results

Functional studies

In all recipients, proteinuria progressed over time (Fig. 1). However, the increase was more pronounced in controls than in treated animals. A proteinuria of more than 30 mg/24 h developed in controls by week 16, whereas in cyclosporin A- and tacrolimus-treated recipients this only occurred after week 20.

The deterioration of kidney function further manifested itself in a decreased creatinine clearance, in parallel with increased serum creatinine levels (Table 1). Suppression of T cells, by both cyclosporin A and tacrolimus, improved kidney function at both time points. These differences reached statistical significance by week 24. At this time point, tacrolimus-treated ($P < 0.01$ vs controls) and cyclosporin A-treated ($P < 0.05$ vs controls) animals had the highest clearance and the lowest serum-creatinine values (Table 1). Parallel to the decline of graft function, mean arterial blood pressure increased from week 16 to week 24 in controls more rapidly than in treated rats (Fig. 2). These differences reached statistical significance ($P < 0.05$) by 24 weeks.

Light microscopy and immunohistology

Histological assessment of glomerulosclerosis, leukocyte infiltration, interstitial fibrosis, and intimal proliferation correlated with kidney function. In controls, more than

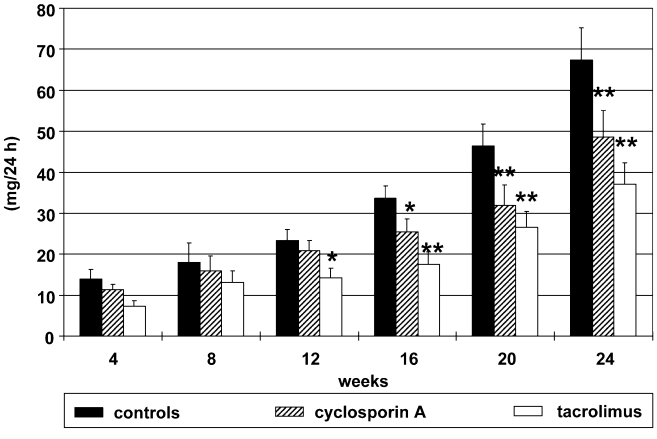

Fig. 1. Urinary protein excretion in 24-h urine samples (U_{prot} [mg/24 h]) (*$P < 0.05$, **$P < 0.01$ vs controls)

Table 1. Creatinine clearance, serum creatinine levels, and glomerulosclerosis (the number of glomeruli with sclerotic lesions expressed as percentage of the total number of glomeruli counted) 16 and 24 weeks after kidney transplantation. Statistical significance within rows was analyzed by means of Fisher's least square distance test [19]. *P* values represent probability of error as a result of Student's *t*-test, comparing the treated group with the control group

Parameter	Weeks	Cyclosporin A, vehicle from day 10		Continuous cyclosporin A		Cyclosporin A, tacrolimus from day 10		*P* value	
		Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	(Cyclosporin A vs controls)	(Tacrolimus vs controls)
Glomerulosclerosis (%)	16	42 ± 1.5	13	30 ± 0.6	15	22 ± 1.6	14	< 0.01	< 0.01
	24	51 ± 2.1	8	39 ± 0.9	10	29 ± 1.5	9	< 0.01	< 0.01
Creatinine clearance (ml/min)	16	1.8 ± 0.1	13	2 ± 0.1	15	1.9 ± 0.1	14	n.s.	n.s.
	24	1.0 ± 0.2	8	1.5 ± ± 0.1	10	1.7 ± 0.1	9	< 0.05	< 0.01
Serum creatinine (mg/dl)	16	1.5 ± 0.2	13	1.3 ± 0.1	15	1.3 ± 0.2	14	n.s.	n.s.
	24	2.1 ± 0.2	8	1.7 ± 0.2	10	1.4 ± 0.1	9	< 0.05	< 0.01

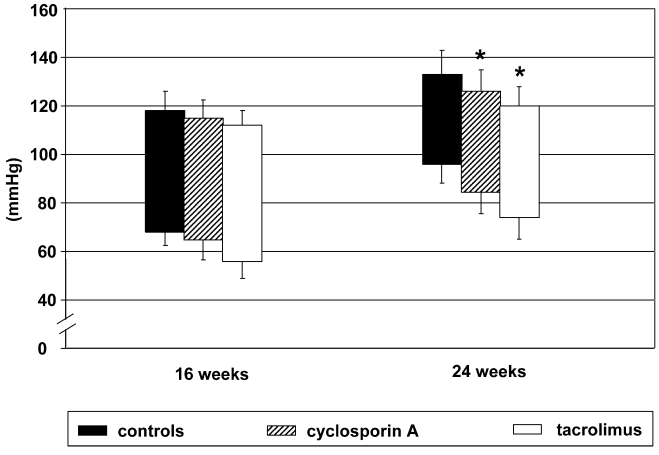

Fig. 2. Systemic blood pressure 16 and 24 weeks after transplantation (*$P < 0.05$ vs controls). *Upper edge of boxes* represents systolic and *lower edge* diastolic intra-aortic blood pressure

40% of glomeruli were sclerosed at week 16. Interstitium and vessel walls in the surrounding of sclerosed glomeruli as well as the glomeruli themselves were infiltrated by large numbers of leukocytes, identified by immunohistological staining as ED-1+ macrophages, and CD5+ T cells; some of them additionally stained positive for LFA-1α and VLA-4α (Table 2). Inhibition of T-cell activation reduced the extent of glomerulosclerosis ($P < 0.01$; Table 1), and the infiltration of ED-1+ macrophages ($P < 0.01$) and, particularly, CD5+ T-cells ($P < 0.001$). Additionally, fewer cells stained positive for LFA-1α and VLA-4 (Table 2).

In controls, cellular infiltration markedly decreased, giving way to extensive interstitial fibrosis and intimal proliferation of the arteries at week 24. In parallel, the number of sclerosed glomeruli increased from week 16 to week 24 (Table 1). Inhibition of T-cell activation ameliorated these changes. Glomerulosclerosis, intimal proliferation, interstitial fibrosis, and leukocyte infiltration was significantly reduced (Tables 1 and 2). In controls, the expression of ICAM-1 and VCAM-1 in the tubulointerstitium and on vessel walls was intense at week 16, but was lower at week 24 (Table 2). Cyclosporin A and tacrolimus reduced the expression of these molecules at both time points.

Polymerase chain reaction

In controls, IL-2 mRNA synthesis paralleled the number of mononuclear cells infiltrating the tubulointerstitium. IL-2 mRNA synthesis was intense at week 16 and had decreased by week 24. Unsurprisingly, both cyclosporin A and tacrolimus significantly ($P < 0.05$ vs controls) inhibited IL-2 synthesis at both time points (Fig. 3).

IL-2Rβ (p70) mRNA levels paralleled IL-2 synthesis: in control animals, receptor synthesis peaked at week 16, and decreased significantly ($P < 0.05$) thereafter. The inhibition of IL-2 synthesis by cyclosporin A and tacrolimus was accompanied by an inhibition of IL-2Rβ mRNA synthesis. However, these differences reached statistical significance ($P < 0.05$) only in the tacrolimus group at week 16 (Fig 3).

TGF-β_1 synthesis was highly upregulated in controls at week 16 (Fig. 4). At week 24, when interstitial fibrosis and glomerular sclerosis had replaced cellular infiltration, TGF-β_1 mRNA was markedly reduced. Continuous suppression of T-cell activation by cyclosporin A and by tacrolimus reduced TGF-β_1 synthesis at weeks 16

Table 2. Immunohistological staining for infiltrating cells and adhesion molecules 16 and 24 weeks after kidney transplantation. Statistical significance within rows was analyzed by means of Fisher's least square distance test [19]. *P* values represent the result of Student's *t*-test, comparing the treated group with the control group (*fv* field of view at 400× magnification)

Parameter	Weeks	Cyclosporin A, vehicle from day 10		Continuous cyclosporin A		Cyclosporin A, tacrolimus from day 10		*P* value	
		Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	(Cyclosporin A vs controls)	(Tacrolimus vs controls)
Macrophages (ED-1, cells/fv)	16	80 ± 8.4	5	60 ± 3.3	5	48 ± 4.4	5	< 0.05	< 0.01
	24	68 ± 4.1	13	47 ± 4.6	10	37 ± 2.3	9	< 0.01	< 0.01
Lymphocytes (CD5, cells/fv)	16	65 ± 6.6	5	45 ± 4.9	5	40 ± 4.4	5	< 0.01	< 0.01
	24	49 ± 3.3	13	33 ± 4.2	10	27 ± 4.6	9	< 0.01	< 0.01
VLA-4α (cells/fv)	16	11 ± 1.6	5	8 ± 0.3	5	7 ± 0.9	5	< 0.05	< 0.01
	24	9 ± 0.8	13	7 ± 0.7	10	5 ± 0.2	9	< 0.01	< 0.01
LFA-1α (cells/fv)	16	8 ± 0.4	5	6 ± 0.9	5	6 ± 0.5	5	< 0.05	< 0.01
	24	7 ± 0.5	13	6 ± 0.4	10	5 ± 0.3	9	< 0.05	< 0.01
ICAM-1 (intensity of 1–4)	16	3.2 ± 0.5	5	2.5 ± 0.6	5	2 ± 0.1	5	n.s.[a]	lt;0.05[a]
	24	2.5 ± 0.6	13	2.2 ± 0.5	10	1.5 ± 0.4	9	n.s.[a]	n.s.[a]
VCAM-1 (intensity of 1–4)	16	3.5 ± 0.6	5	2.5 ± 0.6	5	2.2 ± 0.5	5	n.s.[a]	< 0.05[a]
	24	2.7 ± 0.5	13	2 ± 0.8	10	1.5 ± 0.6	9	n.s.[a]	n.s.[a]

[a]Intensity of staining for ICAM-1 and VCAM-1 was compared by means of Fisher's exact test for ordinal data

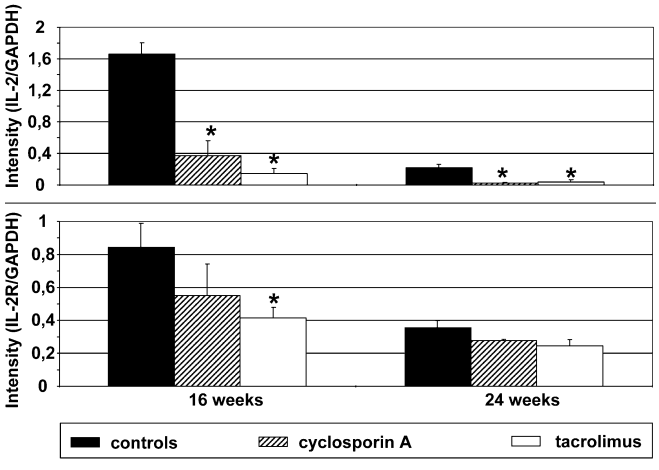

Fig. 3. IL-2 (*upper panel*) and IL-2RB (*lower panel*) mRNA synthesis 16 and 24 weeks after transplantation. Densitometric comparison of IL-2 and IL-2R bands with GAPDH bands (*$P < 0.05$ vs controls)

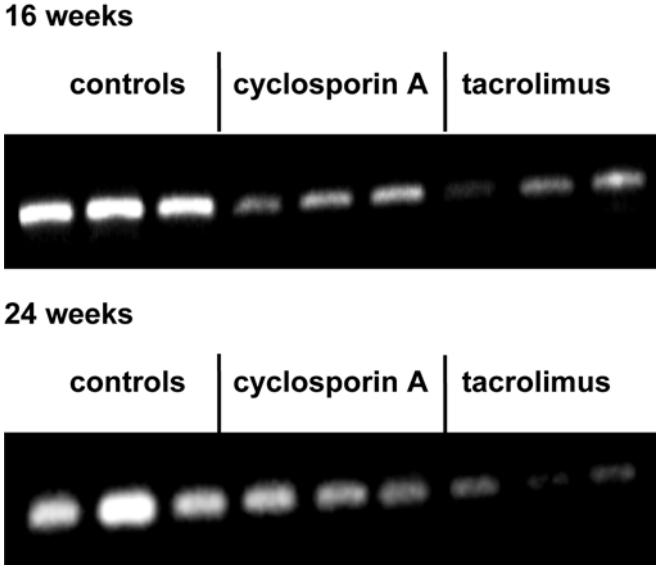

Fig. 4. TGF-β_1 mRNA expression in kidney allograft samples 16 and 24 weeks after transplantation by reverse transcriptase polymerase chain reaction (*RT-PCR*). The PCR products are visualized on agarose gel stained with ethidium bromide

($P < 0.05$ vs controls) and 24 ($P < 0.01$) significantly (Fig. 5).

In contrast to TGF-β_1, PDGF synthesis was at least as highly upregulated at week 24 as at week 16 in controls. While considerably lower expressed in treated rats at week 16 ($P < 0.05$), the synthesis of PDGF increased in both treated groups thereafter (Fig. 6).

A strong correlation was observed between the number of infiltrating CD5+ T cells and IL-2 ($r = 0.84$, $P < 0.01$) as well as TGF ($r = 0.78$, $P < 0.01$) synthesis (Fig. 7).

Discussion

Although it is widely held that CAN is unresponsive to calcineurin inhibition [30], the results presented herein demonstrate a role for T cells in CAN and show that T-cell inhibition might be beneficial in CAN.

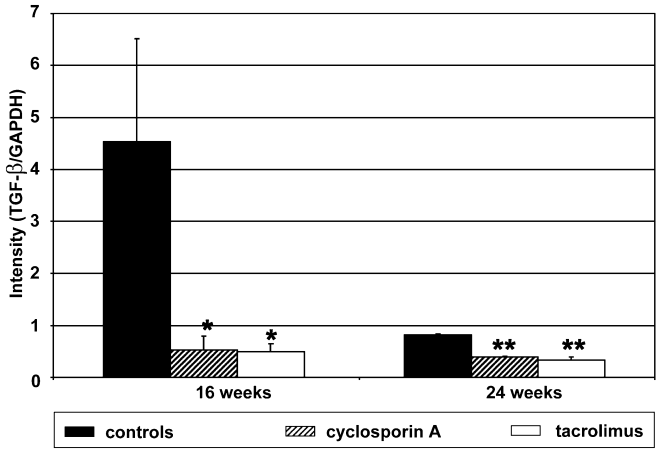

Fig. 5. TGF-β_1 mRNA synthesis 16 and 24 weeks after transplantation. Densitometric comparison of TGF-β_1 bands with GAPDH bands (*$P < 0.05$, **$P < 0.01$ vs controls)

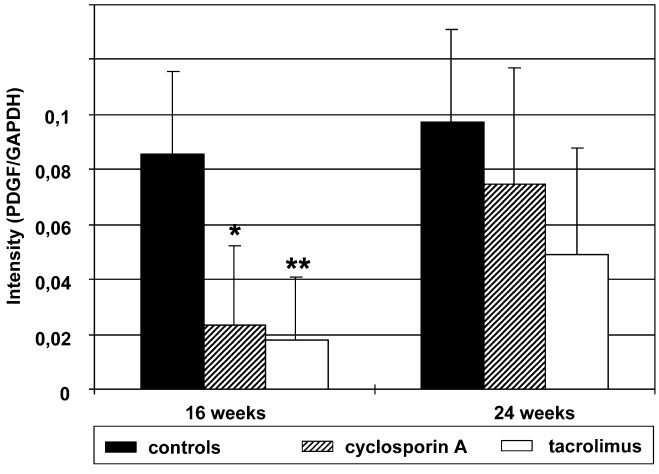

Fig. 6. TGF-β_1 mRNA synthesis 16 and 24 weeks after transplantation. Densitometric comparison of TGF-β_1 bands with GAPDH bands (*$P < 0.05$, **$P < 0.01$ vs controls)

Immunohistochemical analysis of renal transplants undergoing CAN revealed T-cell infiltration [20], with an upregulated ability to adhere to the inflamed endothelium [40]. The reactivity of T cells may spread to a wider range of epitopes after transplantation, demonstrated to be graft destructive [13, 15, 29, 57]. Furthermore, recent studies demonstrated that different strategies eliminating allopeptide-reactive T cells are beneficial [7, 8, 33]. All these observations suggest that alloantigen-specific T cells have an important role in CAN.

CAN may be the result of T-cell-mediated immune reaction to the alloantigen [1, 43], of recurrent, self-limiting acute rejection episodes [28, 36], or of alloantigen-independent events. Injury may activate graft endothelial cells resulting in an unspecific emigration of mononuclear cells into the allograft [38, 48, 50]. As

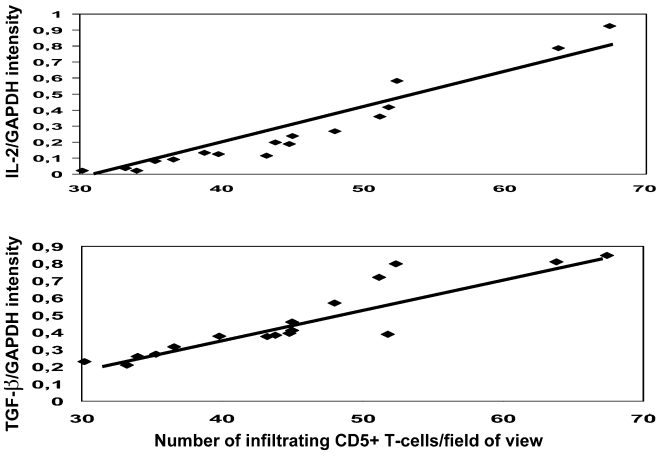

Fig. 7. Correlation of the number of infiltrating CD5+ T-cells with IL-2 synthesis ($r = 0.84$, $P < 0.01$) (*upper panel*) and TGF-β synthesis ($r = 0.78$, $P < 0.01$) (*lower panel*)

lymphocytes are activated during extravasation, all theories suggest an involvement of activated T cells in CAN [14].

In the present rat model, intense cellular infiltration by T cells at week 16 was accompanied by intense synthesis of IL-2 and its receptor. Krams et al. have also demonstrated the presence of IL-2R mRNA in human nephrectomy samples with pathological evidence for CAN [32].

In the present study we tested whether the inhibition of T cells with either cyclosporin A or tacrolimus would ameliorate the process of CAN in renal allografts. The calcineurin inhibitor doses applied in this study were based on previous results in the Fisher-to-Lewis rat model. In this model of CAN, a 10-day cyclosporin-A treatment prevents acute rejection and the grafts survive for more than a year without further immunosuppression. At the time of the experiment we had no data on tacrolimus dosing in rats, and no data on substituting tacrolimus with cyclosporin A for this initial period. Therefore, we performed a pilot study, in which the clinically applied doses of tacrolimus as well as the previously used cyclosporin-A doses appeared to be toxic to our rats. The final doses applied were the highest non-toxic doses. Although there is extensive literature on the comparison of tacrolimus and cyclosporin A, we had no possibilities for a pharmacodynamic analysis of the doses applied in this study. Therefore, we restrain from comparing the effectiveness of these two calcineurin inhibitors. Similarly, although tempted, in the present study on CAN we did not investigate the effectiveness of tacrolimus in controlling acute rejection.

Cyclosporin A and tacrolimus inhibit the T-cell receptor-signalling pathway and thus inhibit T-cell activation-triggered gene expression, including the IL-2 [1, 17, 18, 28] and the IL-2R [35, 60] gene. In the present

study, as expected, both cyclosporin A and tacrolimus inhibited the synthesis of IL-2 as well as IL-2Rβ. This inhibition of T cells was accompanied by a reduced pace of deterioration, as indicated by functional parameters such as proteinuria or creatinine clearance.

A few studies have suggested beneficial effects of a continuous treatment with cyclosporin A on the process of CAN in experimental [31, 34, 58] and clinical [3, 49] transplantation. A study of human cases with biopsy-proven CAN even concluded that too-low an immunosuppression can be one of the factors responsible for CAN [39]. However, none of these studies focuses on the long-term effects of continuous suppression of T-cell activation and proliferation following kidney transplantation. Some clinical studies supported our observations. In biopsy-proven cases of CAN, conversion from azathioprine + prednisolone to cyclosporin A + prednisolone [49] or the addition of cyclosporin A [3] attenuated the rate of decline. Other studies have suggested a beneficial effect of a conversion from cyclosporin A to azathioprine [4, 24].

Our data support the hypothesis that CAN – at least in part – has to do with permanent alloantigen recognition. On the other hand, the involvement of activated T cells may be interpreted as part of the ongoing unspecific inflammatory reaction. Further studies are needed to elucidate this question.

Tissue remodeling processes, such as CAN of renal allografts, may be mediated by locally produced growth factors [47]. TGF-β_1 plays a pivotal role in fibrinogenesis [6]. In biopsies of chronically rejecting kidney allografts [20, 26], the expression of TGF-β_1 was elevated, suggesting that this factor may play an important role in CAN [20, 53]. Although TGF-β is secreted as pro-TGF-β covalently bound to latent associated peptide [26], in our study an intense upregulation of TGF-β mRNA was observed in controls. This upregulation of TGF-β_1 mRNA synthesis had a time-course relationship with the intensity of cellular infiltration. Furthermore, TGF-β synthesis was minimal in immunosuppressed animals, which could explain the reduction of glomerulosclerosis and interstitial fibrosis observed. Cyclosporin A might stimulate the expression of TGF-β_1 in T cells [31], tubule cells [50], or fibroblasts in vitro [59] and in vivo in naive animals. TGF-β is produced by a variety of different T-cell types, including graft-invading macrophages and T cells [20, 46]. Our results suggest that the stimulatory effect of cyclosporine A on local TGF-β production may be minor compared with the inhibition of cellular infiltration and, thus, the elimination of TGF-β-producing mononuclear cells.

There is a growing body of evidence that besides TGF-β, PDGF synthesis is important in tissue remodeling, such as progressive glomerulosclerosis [44]. PDGF is a potent mitogen for mesangial cells [2] and induces mesangial cells to produce TGF-β [31] and fibroblasts to proliferate [44]. Increased expression of PDGF has been documented in a number of fibrotic diseases of the kidney, including renal CAN [5, 11, 37]. In rat renal allotransplantation studies, peak cellular infiltration at week 16 was associated with immunohistochemical staining for PDGF on arterial smooth muscle cells of the graft [5, 23]. Although both cyclosporin A and tacrolimus had a beneficial influence on PDGF synthesis in our study, their influence vanished over time. In treated and untreated animals, PDGF synthesis increased from weeks 16 to 24, despite considerable reduction of infiltrating cells. Since PDGF is produced not only by platelets, but by mesangial cells as well [12], it is suggested that glomerular cell proliferation may become self-perpetuating by an autocrine mechanism involving mesangial cell production of PDGF [12]. Therefore, PDGF may be more important for the later progression of CAN than TGF-β_1. Furthermore, while cyclosporin A and tacrolimus are beneficial in the early phase of CAN, once renal scarring has occurred, alloantigen-independent processes may become dominant in the self-perpetuating process, and therefore there may be no further use of immunosuppression targeting T cells. Kirk et al. also demonstrated that the degree of graft infiltration by T cells and T-cell receptor turnover early (i.e., 2–3 years) after transplantation, correlated with the later development of CAN: functional deterioration, proteinuria, and fibrosis of the graft [30].

In summary, continuous suppression of T-cell activation and proliferation may reduce the pace of CAN of rat kidney allografts.

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