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# Interleukin-2-Dependent Mechanisms Are Involved in the Development of Glomerulosclerosis after Partial Renal Ablation in Rats

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**Key Words**
IL-2 · Tacrolimus · Hyperfiltration · Glomerulosclerosis · Cytokines · Growth factors

**Abstract**
***Background:*** Glomerulosclerosis is a common feature of many end-stage renal diseases. The contribution of cellular immune mechanisms has been implicated in the development of glomerulosclerosis. We investigated whether the inhibition of lymphocyte activation influences this process in an established rat model of renal hyperfiltration. ***Methods:*** After removal of two-thirds of their respective kidney mass, rats were treated with either tacrolimus (0.08 mg/kg/day) or vehicle until the end of the study (n = 10/group). The rats were pair-fed and proteinuria was assessed regularly. Twenty weeks after nephrectomy, creatinine clearance and systemic blood pressure were determined, and kidneys were harvested for morphological, immunohistological and PCR analysis. ***Results:*** In control animals, renal function started to decline from week 12, as indicated by an elevated proteinuria. Interleukin (IL)-2 and IL-2 receptor synthesis was upregulated in control animals and inhibited by tacrolimus treatment. Transforming growth factor-β (TGF-$\beta_1$), platelet-derived growth factor-AA (PDGF-AA) and macrophage chemoattractant protein-1 (MCP-1) mRNA levels were upregulated in control animals, but were significantly lower in immunosuppressed hosts. Additionally, tacrolimus treatment resulted in a significant reduction of proteinuria. Morphological analysis supported these functional results; glomerular sclerosis, tubular atrophy and intimal proliferation were more pronounced in controls than in the tacrolimus group. These morphological parameters were accompanied by reduced infiltration of CD5+ (rat T-cell marker) T cells, ED1+ (rat macrophage marker) macrophages, and less intense staining for laminin and fibronectin. ***Conclusion:*** A continuous treatment with tacrolimus – an inhibitor of lymphocyte proliferation – reduced the pace of glomerulosclerosis in the remnant kidney.



## Introduction

Hyperfiltration-induced glomerular sclerosis is supposedly the common final pathway of many forms of chronic renal diseases [1, 2].

The histological picture of end-stage renal disease often includes glomerular sclerosis, interstitial fibrosis and infiltration of leukocytes [1–3]. A common feature of progressive renal diseases is the continuous loss of func-

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**Table 1.** Body weight, kidney function and systemic blood pressure 20 weeks after nephrectomy

| | Tacrolimus | Controls | p |
|---|---|---|---|
| *Body weight, g* | | | |
| Before nephrectomy | 301.0 ± 6.9 | 301.0 ± 9.1 | NS |
| 4 weeks | 348.6 ± 12.3 | 357.2 ± 15.3 | NS |
| 8 weeks | 386.1 ± 15.8 | 386.3 ± 17.2 | NS |
| 12 weeks | 408.6 ± 16.8 | 412.3 ± 15.7 | NS |
| 16 weeks | 414.8 ± 11.6 | 412.2 ± 14.0 | NS |
| 20 weeks | 406.3 ± 16.4 | 408.7 ± 15.0 | NS |
| Glomerulosclerosis | 16.3 ± 2.75 | 23.9 ± 2.9 | <0.001 |
| Serum creatinine, mg/dl | 1.15 ± 0.12 | 1 ± 0.18 | NS |
| Creatinine clearance, ml/min | 0.10 ± 0.08 | 0.13 ± 0.06 | NS |
| *Blood pressure, mm Hg* | | | |
| Systolic | 167.1 ± 13.7 | 160.9 ± 21.8 | NS |
| Diastolic | 115.3 ± 16.4 | 113.2 ± 9.5 | NS |

tioning nephrons [1, 4]. Hyperfiltration of the remaining glomeruli, hemodynamic changes and an altered metabolic state of the endothelium may activate endothelial cells [4–7]. The activated endothelium is a critical mediator of trafficking and transmigration of inflammatory leukocytes [6]. Previous studies have suggested that inflammatory cytokines [8] and growth factors [9], produced by infiltrating mononuclear cells [10–13] and the activated endothelium [7], might be responsible for the development of glomerulosclerosis and interstitial fibrosis.

The development of interstitial fibrosis has been attributed to local transforming growth factor-β (TGF-β) production [12, 14]. TGF-β induces fibroblast proliferation, synthesis of extracellular matrix proteins such as laminin and fibronectin, and inhibits matrix-degrading enzymes [2]. TGF-β is produced by various cell types, including activated T cells, endothelial cells and particularly macrophages [12, 15]. One of the most important chemokines (chemotactic cytokines) for the recruitment of macrophages is the monocyte chemoattractant protein-1 (MCP-1) [16]. It is produced by mesangial, epithelial and endothelial cells [16]. The recruited macrophages are thought to be one of the main inducers of glomerulosclerosis [11, 12] by producing profibrotic growth factors such as platelet-derived growth factor-AA (PDGF-AA) [17]. PDGF is a strong chemoattractant for fibroblasts, it induces fibroblasts to proliferate and induces mesangial cells to produce TGF-β [2, 17]. Both the PDGF A and B chain can promote smooth muscle cell migration and proliferation and, thus, may play an important role in the pathogenesis of renal interstitial fibrosis [18].

Interleukin (IL)-2, originally called T-cell growth factor, is responsible for the proliferation of T cells upon activation [19, 20], and T-cell activation and proliferation may play an important role in the progression of renal disease [11, 13]. Based on this, our working hypothesis was that not only macrophages, but also infiltrating T cells, as well as their products, determine the progression of glomerulosclerosis. Thus, prevention of lymphocytic activation might reduce the pace of this process.

To test this hypothesis, we investigated the effects of continuous suppression of the IL-2 pathway on cellular infiltration and cytokine production, in a well-established rat model of progressive glomerulosclerosis.

In order to inhibit the IL-2 pathway, we used a daily Tacrolimus® regimen. Tacrolimus affects the proliferation of T cells by inhibiting the expression of IL-2, IL-2 receptor and other lymphokine genes, at the level of mRNA transcription. It selectively reduces IL-2 synthesis by activated helper T cells and inhibits activation of resting T lymphocytes by IL-2 [33].

## Materials and Methods

*Experimental Animals*

Thirteen-week-old male Wistar (WU, RT1[1]) (Charles Rivers, Budapest, Hungary) rats weighing 280–320 g were used throughout the experiments. All animals were kept under standard conditions and received rat chow and water ad libitum. At the time of operation, body weight of the animals averaged 301 ± 7 g in both groups (table 1).

*Renal Ablation*

Under sodium-pentobarbital anesthesia (55 mg/kg i.p.) the right kidney was decapsulated and removed through a midline laparotomy. In the same operative session, one-third of the decapsulated left kidney was resected with scissors, leaving the pelvis and the hilum intact. To stop bleeding, renal vessels were clamped for 5 min [21, 22]. The excised kidney tissue was weighed on an analytic scale. The average reduction of total kidney mass was 66.2 ± 2.8%, ranging from 62.4 to 73.5%.

*Experimental Design*

After renal ablation, rats were divided into two groups (n = 10/group): one group was treated daily with Tacrolimus (0.08 mg/kg/day) (Fujisawa, Japan) [23], while untreated control rats received vehicle alone (n = 10/group). Tacrolimus was suspended in cremophor-ethanol according to the manufacturer's instructions to a final concentration of 0.08 mg/ml and was administered subcutaneously from day 1 postoperative, until the rats were harvested at week 20.

Tacrolimus dosages were determined in a pilot study. Rats chronically receiving 0.32 and 0.16 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, 0.08 mg/kg/day – the highest dose without side effects – was applied.

In an effort to avoid differences in body weight throughout the study, rats were pair-fed (table 1): untreated controls receiving the least amount of food.

Twenty weeks after nephrectomy, serum creatinine was measured and blood pressure determined indirectly in conscious rats using the tail-cuff electrosphygmomanometry [24]. Average blood pressure was calculated based on five readings. Animals were then anesthetized with diethyl ether, bled and the remnant kidney was removed. Samples were snap-frozen in liquid nitrogen for immunohistological staining and PCR analysis or were fixed in formalin (4%) for light microscopy.

*Routine Chemistry*

For protein analysis, 24-hour urine samples were collected every 4 weeks. Urinary protein concentrations were determined by a microspectrophotometer (nephrolometer) at a wavelength of 595 nm after precipitation with 19.4% trichorocetic acid. Serum and urine creatinine concentrations were determined according to Jaffé's method [25] and the optical density was read using an Eppendorf photometer. Creatinine clearance was calculated at the end of the study.

*Antibodies*

Monoclonal antibodies against rat CD5+ T lymphocytes (OX19), macrophages (ED1), ICAM-1 (CD54), VCAM-1 (CD106), VLA-4α (CD49d), and LFA-1α (CD11a) were purchased from Serotec Camon Labor-Service GmbH (Germany).

*Histology and Immunohistology*

To evaluate the extent of glomerulosclerosis, kidney tissues fixed in 4% buffered formalin were embedded in paraffin and stained with hematoxylin and eosin, and periodic acid-Schiff reaction. Glomerulosclerosis was defined as a collapse of the glomerular capillaries, adhesion of the obsolescent segment of Bowman's capsule and the entrapment of hyaline [1]. 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 100 glomeruli was evaluated.

For immunohistology, cryostat sections (4 μm) were fixed in acetone, and stained using alkaline phosphatase-antialkaline phosphatase (APAAP) technique, as previously described [23]. Following this, the sections were counterstained with Mayer's hemalaun (Merck, Darmstadt, Germany). Cells staining positive for antigens 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, laminin, fibronectin) was evaluated in a blinded manner on a scale from 1 to 4, with 1 indicating minimal and 4 intense staining.

*Oligonucleotide Primers*

Rat primers for β-actin, MCP-1, TGF-$\beta_1$, PDGF-AA, IL-2, and IL-2 receptor-α (IL-2Rα) were obtained from Euro Gentec (Belgium).

*Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)*

RNA samples derived from frozen kidney tissue were prepared by using a guanidine isothiocyanate/phenol/chloroform isolation method (RNeasy, Total RNA Isolations Kit; Qiagen GmbH, Germany) [26]. One microgram of total RNA was used for first-strand cDNA synthesis with oligo$(dT)_{12-18}$ as a primer under supplier-recommended conditions (Gibco/BRL). Specific complementary DNA (cDNA) amplification has previously been described [27]. Briefly, specific cDNA products complementary to mRNA for the above listed molecules, were amplified using the polymerase chain reaction (PCR) [27]. A master mix, containing reaction buffer, magnesium chloride, deoxynucleoside triphosphate and Taq DNA polymerase (DIANOVA), was prepared. Specific primers and sample cDNA were added to each master mix. Perkin-Elmer Thermal Cycler (Model 9600) was used for amplification. The amplified PCR product was electrophoresed on an agarose gel, stained with ethidium bromide and gene fragments were visualized by UV light. Quantity of cytokine and growth factor cDNA was estimated by densitometric comparison with β-actin (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 ± SD. Differences between groups were assessed using ANOVA and Student's t-test as appropriate. Nonmetric parameters, such as the intensity of staining for adhesion molecules, were evaluated with Fisher's exact test for ordinal data [28]. A p value of $<0.05$ was considered significant.

## Results

*Functional Studies*

In both control and Tacrolimus-treated rats, proteinuria increased over time (fig. 1). However, the increase was more pronounced in untreated control rats than in treated ones, especially 16 and 20 weeks after nephrectomy ($p<0.05$). On the other hand, at week 20, serum creatinine levels were only slightly elevated in control animals, and creatinine clearance was only slightly reduced (statistically insignificant) in both groups of nephrectomized rats as compared to naive ones. Tacrolimus-treated rats had somewhat lower creatinine clearance and higher creatinine levels than control rats, but these differences were also statistically insignificant (table 1). By this time, all animals had developed a tendency towards higher blood pressure, but the intergroup difference was also statistically insignificant (table 1).

*Light Microscopy and Immunohistology*

In control rats, more than 20% of glomeruli were sclerosed at the time of harvesting. The extent of glomerular sclerosis correlated with the amount of proteinuria (fig. 2). Interstitium and vessel walls in the surrounding of sclerosed glomeruli, as well as the glomeruli themselves, were infiltrated by leukocytes, that were identified by immunohistological staining as CD5+ T cells (fig. 3a) and ED1+ macrophages; some of them additionally positively stained for LFA-1α and VLA-4α (table 2). Inhibition of

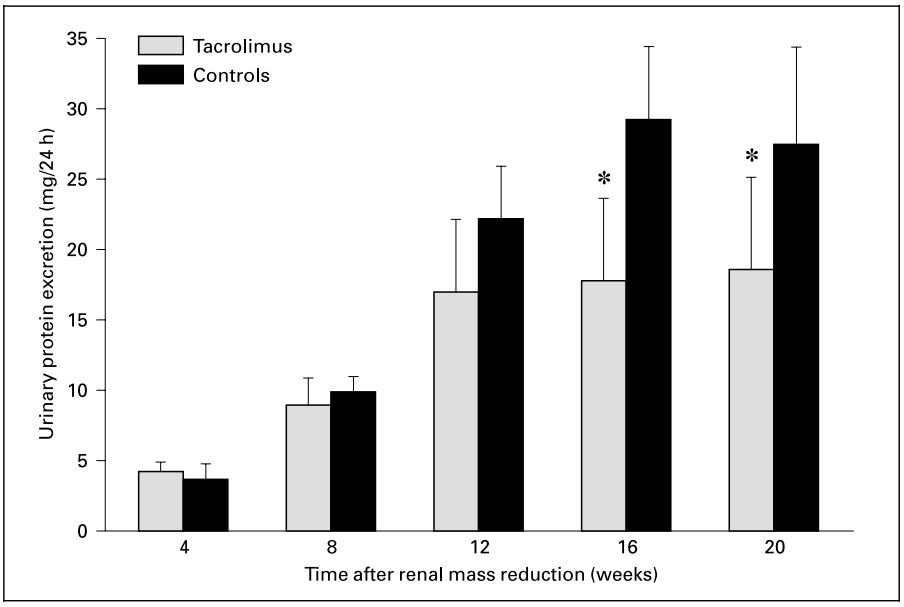


**Fig. 1.** Urinary protein excretion in 24-hour urine samples ($U_{prot}$ (mg)/24 h) (* $p < 0.05$ vs. control rats with ANOVA).

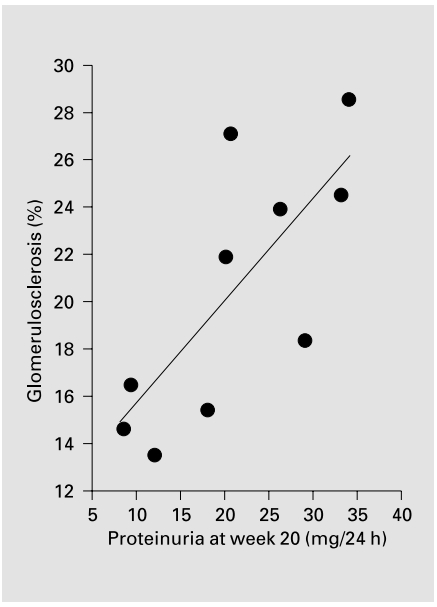


**Fig. 2.** Correlation of glomerulosclerosis with proteinuria 20 weeks after nephrectomy ($r = 0.78$) ($p < 0.001$).

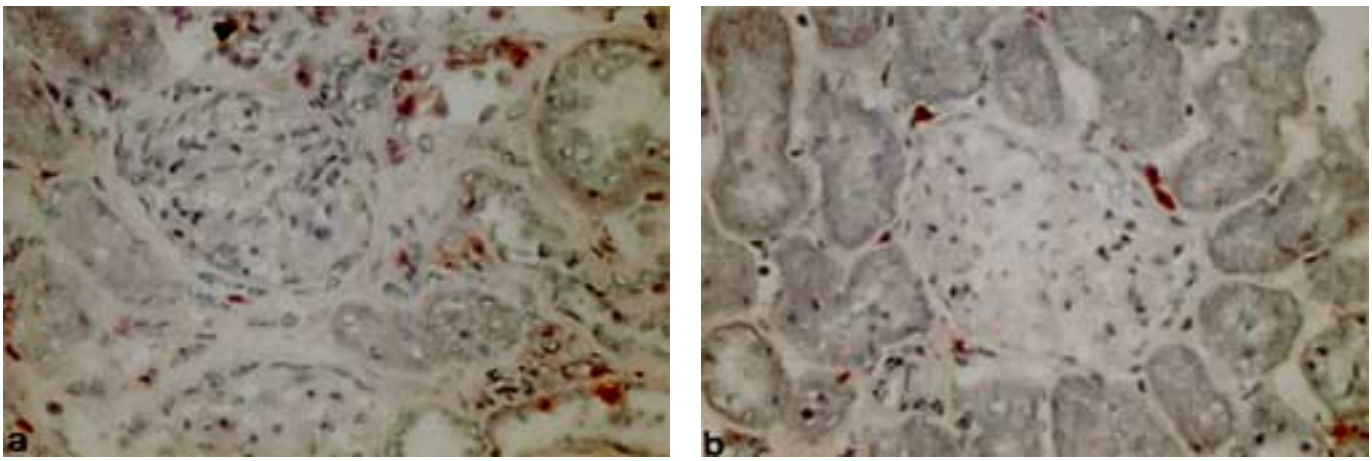


**Fig. 3.** Immunohistological staining for CD5+ T cells (red-brown) from kidneys of control rats (**a**) and from Tacrolimus-treated ones (**b**), 20 weeks after nephrectomy.

lymphocyte proliferation by Tacrolimus reduced the extent of glomerulosclerosis ($p < 0.01$; table 1). This was accompanied by a reduced number of infiltrating CD5+ T lymphocytes ($p < 0.05$) (fig. 3b) and ED1+ macrophages ($p < 0.05$; table 2) and fewer LFA-1α- and VLA-4α-positive stained cells. The number of infiltrating macrophages correlated strongly with IL-2 mRNA synthesis (fig. 6d).

Glomerulosclerosis was associated with tubular atrophy, intimal proliferation of the arteries and interstitial fibrosis. Control rats intensely expressed extracellular matrix proteins (laminin and fibronectin) as well as adhesion molecules (ICAM-1 and VCAM-1) in the tubulointerstitium, and within vessel walls. Inhibition of lymphocyte proliferation ameliorated these changes. Intimal proliferation of arteries and interstitial fibrosis was notably

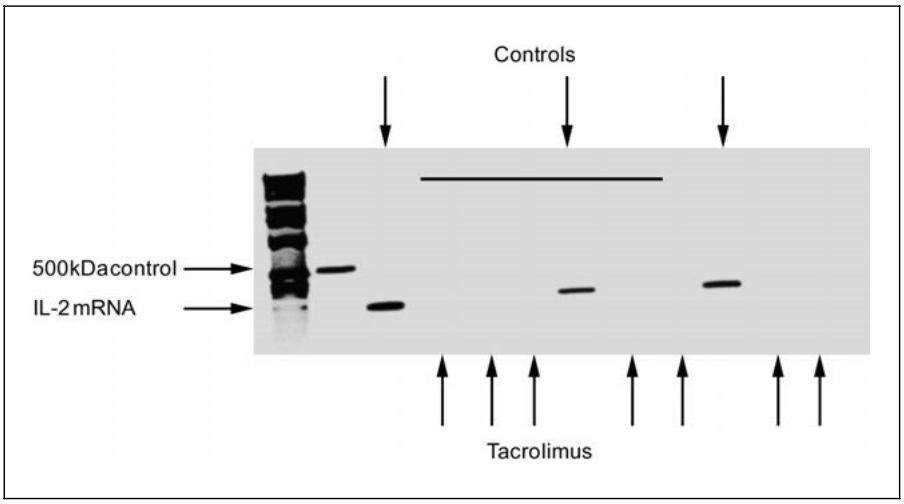


**Fig. 4.** IL-2 mRNA expression in remnant kidney samples 20 weeks after nephrectomy, as detected by RT-PCR. The PCR products were visualized on agarose gel by staining with ethidium bromide.

**Table 2.** Infiltration by macrophages and lymphocytes, expression of adhesion molecules on the surface of leukocytes and staining for extracellular matrix proteins 20 weeks after nephrectomy

| | Tacrolimus | Controls | p |
|---|---|---|---|
| *Cells/field view* | | | |
| Macrophages (ED1) | 16 ± 4.4 | 25 ± 6.2 | <0.05 |
| Lymphocytes (CD5) | 14 ± 3.0 | 20 ± 6.9 | <0.05 |
| VLA-4α | 4 ± 0.5 | 7 ± 0.9 | <0.05 |
| LFA-1α | 3 ± 0.8 | 5 ± 1.2 | <0.05 |
| *Intensity of staining (1–4)* | | | |
| ICAM-1 | 1.6 ± 0.5 | 1.9 ± 0.8 | NS |
| VCAM-1 | 1.75 ± 0.7 | 2 ± 0.8 | NS |
| Laminin | 1.5 ± 0.5 | 2.3 ± 0.5 | <0.05 |
| Fibronectin | 2.25 ± 0.5 | 2.75 ± 0.7 | <0.05 |

reduced and staining for laminin and fibronectin was less intense. The intensity of staining for ICAM-1 and VCAM-1 was also less pronounced, but this difference did not reach statistical significance (table 2).

*Polymerase Chain Reaction*

IL-2 and IL-2Rα mRNA synthesis was increased in control rats at week 20, correlating to the number of leukocytes infiltrating the tubulointerstitium. As expected, Tacrolimus significantly inhibited both IL-2 and IL-2Rα synthesis (fig. 4, 5a).

The synthesis of TGF-$\beta_1$ mRNA was highly upregulated in the control group at week 20 (fig. 5b). Continuous suppression of IL-2-activated T-lymphocytic prolifera-

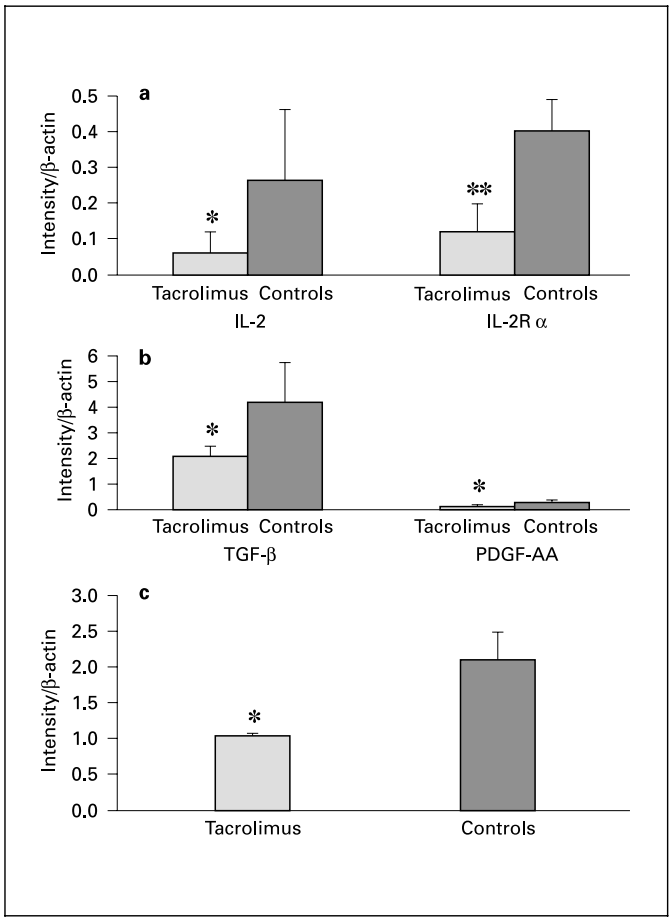


**Fig. 5.** Synthesis of IL-2, IL-2Rα (**a**), TGF-$\beta_1$, PDGF-AA (**b**) and MCP-1 mRNA (**c**) 20 weeks after nephrectomy as determined by RT-PCR. Densitometric comparison of cytokine and growth factor mRNA bands with β-actin bands (* $p < 0.05$, ** $p < 0.01$ vs. control rats).

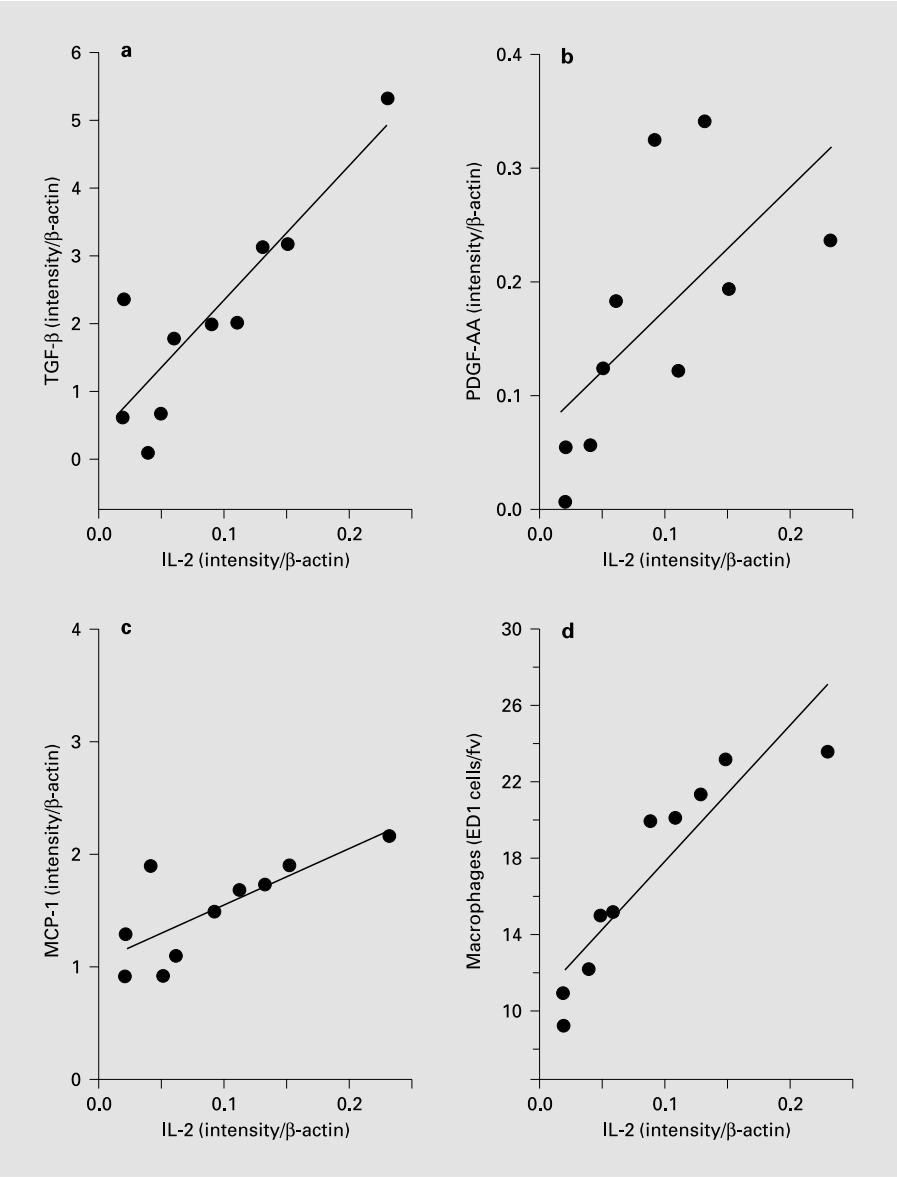


**Fig. 6.** Correlation of (**a**) TGF-$\beta_1$ ($r = 0.88$) ($p < 0.001$), (**b**) PDGF-AA ($r = 0.64$) ($p < 0.05$), (**c**) MCP-1 ($r = 0.76$) ($p < 0.01$) synthesis and (**d**) macrophage count ($r = 0.84$) ($p < 0.01$) with IL-2 synthesis 20 weeks after nephrectomy.

tion by Tacrolimus significantly reduced TGF-$\beta_1$ mRNA synthesis.

PDGF-AA synthesis was also upregulated in control rats, but not in treated animals (fig. 5b). The synthesis of both growth factors correlated significantly with IL-2 production (fig. 6a, b).

In parallel to the number of infiltrating mononuclear cells, the synthesis of MCP-1 was significantly reduced in Tacrolimus-treated animals, as compared to untreated controls (fig. 5c). MCP-1 mRNA synthesis correlated strongly with IL-2 synthesis, both in treated and control rats (fig. 6c).

## Discussion

Progressive glomerular sclerosis is the terminal pathway of many forms of chronic renal diseases [1, 13], such as chronic rejection of renal allografts [29], diabetic [13] or hypertensive [30] nephropathy. The underlying pathological processes remain unclear. Nephron loss due to kidney damage may induce hyperfiltration of the remaining glomeruli [3, 5]. The increased mechanical stress may activate endothelial cells [2], which in turn express increased levels of adhesion molecules, thus allowing mononuclear cells to attach and infiltrate into the kidney tissue

[7, 16, 31]. Various autoimmune and non-antigen-dependent glomerular diseases were associated with an influx of leukocytes into a diseased kidney [11, 16]. It is important to emphasize that lymphocytic infiltration is a prominent finding, not only in autoimmune kidney diseases, but also in other progressive renal diseases, such as hypertensive or diabetic nephropathy, or in the rat renal mass reduction model of progressive glomerulosclerosis. In a non-antigen-dependent model of progressive glomerulosclerosis, the number of infiltrating T cells and macrophages significantly correlated with the progression of the disease [2, 11] and predicted long-term prognosis [2]. In another rat model of glomerulosclerosis, the infiltrating cells expressed activation markers such as the IL-2R [2, 32]. These data suggest an important role of lymphocytes and the involvement of the IL-2 pathway in non-antigen-dependent progressive glomerulosclerosis.

Quiescent human T cells do not express either IL-2 nor the IL-2Rα chain (the p55 molecule) mRNA, in culture [33–35] or in vivo [19]. IL-2 and IL-2Rα are synthesised upon T-cell activation [36]. In the present study, both IL-2 and IL-2Rα were upregulated in control rats at week 20, i.e. when leukocyte infiltration was intense.

A few studies have reported some beneficial effects of different immunosuppressive regimens on the pace of glomerulosclerosis. Azathioprine had inhibitory effects on the hyperplastic response in uninephrectomized mice [37], and prednisolone decreased the interstitial leukocyte infiltration and improved renal function in two non-antigen-dependent rat models of progressive glomerular sclerosis [11, 32]. Fujihara et al. [38] recently reported that mycophenolate mofetil – an inhibitor of lymphocyte proliferation – attenuated renal injury in the rat remnant kidney. However, none of these studies had focused on the involvement of the IL-2-dependent T-lymphocyte proliferation and its interaction with cytokine and growth factor synthesis.

As its most characteristic effect, Tacrolimus inhibits T-cell receptor signal transmission and, thus, inhibits T-cell activation-triggered gene expression, including the IL-2 [19, 20] and the IL-2R [20, 39] gene. In the present study, Tacrolimus reduced the synthesis of IL-2 and IL-2Rα, as well as lymphocytic infiltration.

Infiltrating macrophages and T lymphocytes release growth factors such as TGF-β and PDGF [12, 14, 15]. These locally produced growth factors are thought to mediate tissue remodeling processes, such as chronic fibrosis and glomerulosclerosis [40].

In different models of progressive glomerulosclerosis, the expression of TGF-$\beta_1$ – an important regulator of mesangial matrix protein synthesis [41] – was elevated [7, 12, 14]. Coimbra et al. [42] demonstrated temporal association between glomerulosclerosis, interstitial fibrosis, intense mononuclear cellular infiltration and TGF-β expression in a renal ablation rat model. In the present study, TGF-$\beta_1$ synthesis was upregulated in control, but was minimal in immunosuppressed animals. This could explain the observed reduction of glomerulosclerosis and interstitial fibrosis.

Another important factor for tissue remodeling is PDGF [43], probably the most potent mitogen for mesangial cells [44]. PDGF has been hypothesized to be responsible for accumulation of extracellular matrix proteins (laminin, fibronectin) in the remnant kidney in a rat model [45, 46]. Increased expression of PDGF mRNA has been documented in a variety of renal diseases [44, 47] including the rat renal ablation model [45, 48]. In our study, PDGF-AA synthesis was upregulated in control rats. This was associated with an intense immunohistological staining of laminin and fibronectin. Kovács et al. [49] have demonstrated in vitro that IL-2 induces TGF-β and PDGF expression in macrophages. Therefore, inhibition of IL-2 synthesis by Tacrolimus may decrease growth factor synthesis either directly or indirectly by diminished cellular infiltration.

Profibrotic growth factors such as TGF-β and PDGF are thought to be produced mainly by macrophages [9]. Floege et al. [47] suggested that glomerulosclerosis and monocyte/macrophage infiltration in the remnant kidney may be preceded by an initial proliferation of mesangial cells, associated with increased PDGF expression. The proliferating mesangial cells produce MCP-1. In the rat remnant kidney model, MCP-1 was localized predominantly in areas of focal glomerulosclerosis and its synthesis was elevated in comparison to naive control rats [50–52]. In our model, MCP-1 was intensely synthesized in control rats. Inhibition of IL-2 synthesis significantly inhibited MCP-1 mRNA synthesis, in parallel to a reduced infiltration of macrophages. Both MCP-1 synthesis and macrophage infiltration significantly correlated to IL-2 synthesis. As IL-2 receptors were detected on the surface of macrophages [2, 19, 36], it is possible that inhibition of the IL-2 pathway also interferes with macrophage activation in progressive glomerular sclerosis. However, the reduced macrophage count may be a consequence of reduced T-cell activity, and reduced T-cell interaction with macrophages. Saito and Atkins [11] suggested that although macrophages might be the ultimate effectors of glomerulosclerosis, T cells might direct monocyte activation and thus have an important role in the progression of disease [11].

Serum creatinine and creatinine clearance values in our study were within the normal range, and blood pressure values exceeded normal values only slightly in all animals. These, almost normal, functional parameters paralleled the mild sclerosis observed. Floege at al. [45] demonstrated that glomerular cell proliferation was transient in an ablation model of progressive glomerular sclerosis. Thus, an inhibition of the IL-2-activated T-lymphocyte proliferation pathway might be beneficial at an early stage of progressive glomerulosclerosis, when cellular infiltration dominates.

A known side effect of Tacrolimus in toxic doses is a constriction of the afferent renal artery [53], and a consequent decrease in renal plasma flow and glomerular filtration [54] which might explain the observed slight differences in creatinine clearance and blood pressure between the groups. The above study [54] described that Tacrolimus was able to reduce hyperfiltration if given acutely in a dose of 3 mg/kg b.w., or chronically if given 0.6 mg/kg b.w. In our pilot study for the appropriate dose of Tacrolimus, 0.32 and 0.16 mg/kg b.w. were found toxic, therefore in the present study the applied dose was 0.08 mg/kg b.w. The hemodynamic effects in this dose are not known, but taking into consideration the new study demonstrating that an inhibition of lymphocytic proliferation by mycophenolate mofetil slowed down the progression of renal deterioration in subtotally nephrectomized rats, the IL-2-inhibiting effect is more probable to be responsible for the observed beneficial effects than the hemodynamic effects of Tacrolimus.

In conclusion, inhibiting the proliferation of IL-2-activated T lymphocytes by Tacrolimus decreased the pace of progressive glomerulosclerosis in this renal ablation rat model. This stresses the importance of lymphocytes in the development of renal diseases.

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