CYCLOSPORINE A AND AZATHIOPRINE ARE EQUIPOTENT IN CHRONIC KIDNEY ALLOGRAFT REJECTION[1]

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## Outline

Abstract

Chronic rejection is the major cause of graft loss after kidney transplantation. Various immunosuppressive protocols have been used to ameliorate this process. We investigated whether cyclosporin A- (CyA) or azathioprine- (Aza) based immunosuppression is better able to slow the progression of chronic rejection. Fisher kidneys were transplanted into bilaterally nephrectomized Lewis rats. Recipients received CyA (1.5 mg/kg/day, s.c.) for 10 days, and were treated from day 11 with either CyA (1.5 mg/kg)+pred (0.15

mg/kg) (C+P), Aza (2 mg/kg)+pred (A+P), vehicle+pred (P), or vehicle alone (controls) (n=8/group). Proteinuria was regularly assessed and grafts were harvested for morphological, immunohistological, and molecular biological analysis at week 24. By week 12 proteinuria had increased to significant levels. At week 24, proteinuria was significantly lower and creatinine clearance was significantly higher in C+P and A+P, than in P or controls. Morphological analysis supported these functional results: at week 24, glomerulopathy, tubular atrophy and intimal proliferation (as assessed according to the BANFF score) were less pronounced in C+P and A+P, as compared with P or controls. These morphological parameters were accompanied by a reduced infiltration of ED-1+ macrophages and CD-5+ T lymphocytes. In P or controls the synthesis of IL-2R[alpha] mRNA was markedly elevated at this time. In parallel to the reduced cellular infiltration, IL-2R[alpha] mRNA expression was markedly inhibited, both, in C+P and A+P. There were no significant differences between C+P and A+P regarding the parameters studied. In conclusion, both C+P and A+P reduced the infiltration of activated T lymphocytes, and the pace of chronic kidney allograft rejection. The outcome of C+P and A+P based therapy did not differ significantly.

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Despite a 1-year graft survival of 80-90% in most transplant centers, renal transplantation has not reached its potential as a permanent treatment of chronic renal failure (1). Chronic rejection is the predominant reason for late graft loss (2). Although the introduction of cyclosporin A has improved short-term graft survival, the mean half-life of cadaveric kidney grafts did not change over the last two decades (3, 4). The rate of attrition observed in cyclosporin A-treated patients closely mirrors that of the chronic decline common to azathioprine-treated ones (5, 6). The factors influencing this process are ill defined.

In chronic rejection, the involvement of both alloantigen-dependent and independent factors has been hypothesized (2). The extent to which these mechanisms participate in the progression is not yet understood. As IL-2R positive lymphocytes were constantly detected in chronically rejecting kidneys (7, 8), they may determine the pace of chronic rejection (3, 4) stressing the importance of alloantigen-dependent events.

Both cyclosporin A- and azathioprine-based therapy aims to suppress immune responses to the alloantigen (5). However, the effect on long-term outcome of these drug regimens has not been established (9).

Some clinical studies suggested beneficial effects of conversion from azathioprine- to cyclosporin-based therapy (10, 11). Other studies suggested a beneficial effect of a conversion from cyclosporin A to azathioprine (12, 13). Pabico et al. (4) expressed some concerns regarding the use of cyclosporin A in chronic renal allograft rejection in general.

The long term use of prednisolone has also been recently discussed. Opelz (14) reported that induction therapy with a steroid-free cyclosporin regimen, as well as steroid free maintenance therapy resulted in significantly better 5-year graft and patient survival, than steroid containing regimens (14). However, it has to be considered that only those patients who have a stable graft function are likely to be weaned off prednisolone. Therefore it is safe to assume that the patients reported are a positive selection.

To evaluate the necessity of continuous cyclosporin, azathioprine, or prednisolone therapy, we studied whether the pace of chronic kidney allograft rejection can be influenced by either drug regimens.

MATERIALS AND METHODS

Experimental animals.

Naive male inbred Lewis (LEW) and Fisher (F-344) rats weighing 190-250 g were used throughout the experiment. All animals were obtained from Charles River, Munich, Germany, housed under standard conditions, and received rat food and water ad libidum.

Renal transplantation.

Fisher rats served as donors and Lewis rats as recipients. Transplantation was performed as previously described (15). Briefly, the left donor kidney was perfused with 4°C cold ringer lactate, removed, and positioned orthotopically into the recipient, whose renal vessels had been isolated, clamped, and the left native kidney removed. End-to-end anastomosis of renal artery, vein, and ureter were performed using 10-0 prolene sutures. Total graft ischemia was less than 30 min. To prevent infectious complications during the perioperative phase, rats received 20 mg/kg Cephtriaxone (Rocephine) daily during the first 10 postoperative days, at which time the right native kidney was removed.

Experimental design.

Rats received 1.5 mg/kg body weight cyclosporin A (Calbiochem Biochemicals GmbH, Bad Soden, Germany) for the first 10 postoperative days to prevent an initial episode of acute rejection. After day 10, animals were divided into two treatment and two control groups (n=8/group) and received either cyclosporin A (1.5 mg/kg/day)+prednisolone (0.15 mg/kg/day) (C+P), azathioprine (2 mg/kg/day)+prednisolone (0.15 mg/kg/day) (A+P), vehicle+prednisolone (0.15 mg/kg/day) (P), or vehicle alone (controls) on a daily basis from day 11 until the end of the study. Additionally, we added a group of sham operated animals which were treated with cyclosporine A for the first 10 days after operation. Azathioprine and prednisolone were suspended in NaCl (0,9%) solution, and cyclosporin was suspended in

cremophor-ethanol, according to the manufacturer's instructions. Cyclosporin A was administered s.c. throughout the study, whereas azathioprine and prednisolone were administered i.p. for the first month, and s.c. thereafter.

To exclude the influence of body weight on the extent of proteinuria, body weight was matched at the time of operation. Weight differences between the groups, did not reach statistical significance throughout follow-up.

After 24 weeks, rats were anesthetized, and intraaortic blood pressure was measured using a DPT 3003-S/3cc arterial transducer (Peter von Berg Medizintechnik GmbH, 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 analysis, or fixed in buffered formalin (4%) for light microscopy.

Routine chemistry.

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

Antibodies.

Monoclonal antibodies against CD5+ rat T lymphocytes (OX19), macrophages (ED-1), intercellular adhesion molecule-1 (ICAM-1) (CD-54), [alpha] integrin of very late activation protein-4 (VLA-4[alpha]) (CD49d), isoform 1 of transforming grwoth factor-[beta] (TGF-[beta]), and [alpha] chain of the leukocyte function associated antigen-1 (LFA-1[alpha]) (CD11a) were purchased from Serotec Camon Labor-Service GmbH (Wiesbaden, Germany).

Histology and immunohistology.

For histology, kidney tissues were fixed in 4% buffered formalin, embedded in paraffin, and stained with hematoxylin/eosin and periodic acid-Schiff to evaluate the extent of glomerular sclerosis and interstitial fibrosis. Glomerulosclerosis, mesangial matrix increase of glomeruli, tubular atrophy, interstitial inflammation and fibrosis, and vascular intimal proliferation, were quantified according to the chronic allograft damage index (CADI) (16). Slides were scored in a blinded fashion, semiquantitatively from 0 to 3 (0=no histopathological change, 1: <25%, 2: 25–50%, 3: >50% of the analyzed structures show pathological

changes) ([16](#)). Furthermore, the extent of glomerulosclerosis was evaluated. Glomerulosclerosis was defined as a collapse of the glomerular capillaries, adhesion of the obsolescent segment of Bowman's capsule, and the entrapment of hyaline ([17](#)). 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 CADI score as well as for glomerulosclerosis.

For immunohistology, cryostat sections (4 μm) were fixed in acetone, and stained individually using alkaline-phosphatase-anti-alkaline-phosphatase (APAAP) technique as previously described ([18](#)) and counterstained with Mayer's hemalaun (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 400x magnification. The intensity of tissue staining [intercellular adhesion molecule-1 (ICAM-1)] was evaluated in a blinded manner on a scale from 1–4, with 1 indicating minimal and 4 intense staining.

Reverse transcriptase polymerase chain reaction (RT-PCR).

RNA samples derived from frozen kidneys were prepared by using a guanidine isothiocyanate/phenol/chloroform isolation method (RNeasy, Total RNA Isolations Kit; Qiagen GmbH, Germany) ([19](#)). One μg of total RNA was used for first-strand cDNA synthesis with oligo(dT)$_{12-18}$ as primer under supplier recommended conditions (GIBCO/BRL). Specific complementary cDNA amplification has been previously described ([19](#)). Briefly, specific cDNA products complementary to mRNA sequences of rat IL-2 receptor [alpha] (p55) (CD-25), TGF-[beta]$_1$, the A chain of platelet-derived growth factor (PDGF) and [beta]-actin (Euro Gentec, Köln, Germany) were amplified using the polymerase chain reaction (PCR, 40 cycles) ([19](#)). 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, stained with ethidium bromide and gene fragments were visualized by UV light. Quantity of IL-2 receptor, TGF-[beta]$_1$, and PDGF-A cDNA was estimated by densitometric comparison with [beta]-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±SEM. Differences between the four groups were assessed using analysis of variation and probability values between treated and control groups were calculated with Student's t test

as appropriate. Nonmetric parameters such as the CADI score, and the intensity of staining for ICAM-1 were evaluated with Fisher's exact test for ordinal data (20). P <0.05 was considered significant.

RESULTS

Functional studies.

In all recipients, proteinuria progressed over time (Fig. 1). Kidney function was preserved longer in C+P and A+P than in P and controls. Proteinuria of more than 25 mg/24 hr developed in controls and P by week 16, in A+P-treated recipients by week 20 and in C+P treated ones by 24 weeks. By 24 weeks the reduction of proteinuria, both, in the C+P as well as in the A+P group had reached statistcal significance (P <0.05). P alone had no significant effect.
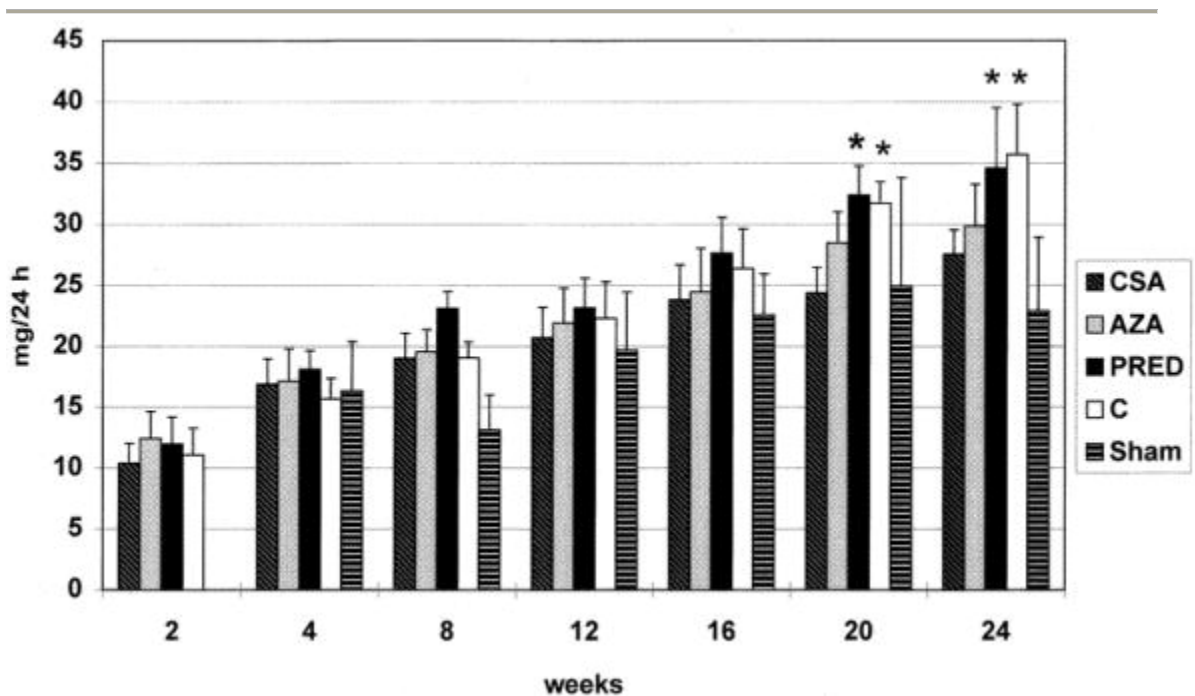


Figure 1. Urinary protein excretion in 24 h urine samples. ($U_{prot}$ (mg)/24 hr) (*P <0.05 vs. controls).

The deterioration of renal function further manifested in a decreased creatinine clearance, in parallel to increased serum creatinine levels 24 weeks after transplantation. Immunosuppression both by C+P and A+P improved kidney function: creatinine clearance was significantly (P <0.05) higher, and serum creatinine levels were significantly (P <0.05) lower, in both the C+P and the A+P group as compared with P and controls (Table 1).

## Table 1

TABLE 1. Kidney function and morphology: Creatinine clearance, serum creatinine levels, glomerulosclerosis (the number of glomeruli with sclerotic lesions expressed as percentage of the total number of glomeruli counted), chronic allograft damage index (CADI) score (*16*), and mean arterial blood pressure (MAP) 24 weeks after kidney transplantation.

| | C+P | A+P | P | Sham operated | Controls |
|---|---|---|---|---|---|
| Crea. clearance (ml/min) | $1.3\pm0.09^a$ | $1.4\pm0.06^a$ | $1.2\pm0.05$ | $1.6\pm0.02$ | $1.2\pm0.07$ |
| Serum crea. (mg/dl) | $0.9\pm0.04^a$ | $0.9\pm0.03^a$ | $1.0\pm0.05$ | $0.7\pm0.02$ | $1.0\pm0.02$ |
| Glomerulosclerosis (%) | $14.4\pm1.3^a$ | $15.3\pm1.6^a$ | $17.8\pm0.9$ | $2.3\pm0.01$ | $21.9\pm3.3$ |
| CADI (score: 0–12)# | $2\pm0.4^a$ | $2.3\pm0.3^a$ | $4.8\pm0.6$ | $1.75\pm0.3$ | $3.6\pm0.5$ |
| MAP (mmHg) | $108\pm3.9^a$ | $106\pm4.9^a$ | $124\pm3.3$ | $88.25\pm6.3$ | $122\pm3.1$ |

Statistical significance was analyzed with analyses of variation and Student's *t* test. *P* represents probability of error as a result of comparing the treated group with the control group.

[a] The CADI sore was evaluated statistically with Fisher's exact test for ordinal data (*20*).

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In parallel to the decline of graft function, rats were slightly hypertensive at week 24. Mean arterial blood pressure was significantly lower (P <0.05) in C+P and A+P as compared to P and controls ([Table 1]). Sham-operated animals did not develop any significant changes over the period observed.

Light microscopy, and immunohistology.

Histological assessment of glomerulosclerosis, correlated with kidney function. In controls 21.9±3.3% of the glomeruli showed sclerotic lesions. Both C+P and A+P treatment significantly (P <0.05) lowered the glomerulosclerosis index. In P the percentage of sclerotic glomeruli did not differ significantly from controls ([Table 1]).

The additional assessment of leukocyte infiltration, interstitial fibrosis and intimal proliferation according to the CADI score gave similar results. The CADI score in controls was 3.6±0.5. Average glomerulopathy

score (sclerosis and mesangial matrix proliferation) was 1±0.1, tubulopathy score was 1.1±0.09, vascular intimal proliferation score was 1.1±0.2, and interstitial inflammation and fibrosis score averaged 0.4±0.09. Both C+P and A+P treatment significantly lowered the CADI score as compared to controls, whereas P had no effect. Sham-operated animals did not develop any significant changes over the period observed.

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 ED1+ macrophages and CD5+ T lymphocytes; some of them additionally stained positive for LFA-1[alpha] and VLA-4[alpha] (Table 2). ICAM-1 expression was intense in the tubulointerstitium and on vessel walls in controls (Table 2). C+P and A+P treatment significantly lowered the infiltration of T lymphocytes (P <0.05). Interestingly we observed only a minor influence on the number of ED1+ macrophages, and adhesion molecule positive cells, or the intensity of staining for ICAM-1. We observed a lower number of cells staining positive for TGF-[beta] in A+P-treated animals as compared to controls. Results were similar to controls in the other groups (Table 2).

## Table 2

### TABLE 2. Immunhistological staining for infiltrating cells, adhesion molecules, and TGF-$\beta$ 24 weeks after kidney transplantation

| | C+P | P | A+P | P | P | P | Controls |
|---|---|---|---|---|---|---|---|
| Macrophages (ED1; cells/fv) | 33.5±4.7 | NS | 32.6±6.3 | NS | 40.4±4.3 | NS | 40.4±4.2 |
| Lymphocytes (CD5; cells/fv) | 34.1±3.6 | <0.05 | 36.3±3.9 | <0.05 | 49.3±5.7 | NS | 49.2±5.5 |
| VLA-4$\alpha$ (cells/fv) | 3.6±0.6 | NS | 3.6±0.4 | NS | 4.4±0.7 | NS | 4.1±0.7 |
| LFA-1$\alpha$ (cells/fv) | 2.8±0.4 | NS | 2.8±0.5 | NS | 2.8±0.4 | NS | 3.9±0.7 |
| ICAM-1 (intensity: 1–4)[a] | 1.4±0.2 | NS | 1.7±0.4 | NS | 2.1±0.2 | NS | 2±0.3 |
| TGF-$\beta$ (cells/fv) | 10.5±2.5 | NS | 2.7±0.6 | <0.05 | 12.6±6.9 | NS | 9.6±2.5 |

Statistical significance was analyzed with analyses of variance and Student's $t$ test. P represents probability of error as a result of Student's $t$ test, comparing the treated group with the control group.

[a] Intensity of staining for ICAM-1, VCAM-1, and LFA-1 was evaluated statistically with Fisher's exact test for ordinal data (20). (fv.: field of view at 400× magnification.)

Table 2. Immunhistological staining for infiltrating cells, adhesion molecules, and TGF-[beta] 24 weeks after kidney transplantationStatistical significance was analyzed with analyses of variance and Student's t test. P represents probability of error as a result of Student's t test, comparing the treated group with the control group.[a] Intensity of staining for ICAM-1, VCAM-1, and LFA-1 was evaluated statistically with Fisher's exact test for ordinal data (20). (fv.: field of view at 400× magnification.)

PCR.

Quiescent human T lymphocytes do not express IL-2 receptor [alpha] chain (CD25) mRNA in culture ([21, 22](#)) or in vivo ([5](#)). In controls, the mRNA synthesis of IL-2R [alpha] was up-regulated. In parallel to the significantly reduced infiltration of T lymphocytes by both C+P and A+P treatment, the IL-2 R[alpha] mRNA was undetectable in these animals ([Fig. 3](#)). P treatment alone did not reduce IL-2 R[alpha] mRNA levels statistically significant as compared to controls ([Table 3](#)). There was a strong correlation (r=0.98, P <0.001) between IL-2 R[alpha] mRNA levels, and the number of infiltrating CD 5+ T lymphocytes in P and controls ([Fig. 2](#)).

## Table 3

**TABLE 3. IL-2R$\alpha$ (CD 25), TGF-$\beta_1$, and PDGF-A mRNA synthesis 24 weeks after transplantation**

| Intensity/$\beta$actin | C+P | P | A+P | P | P | P | Controls |
|---|---|---|---|---|---|---|---|
| IL-2R$\alpha$ (CD25) | 0 | | 0 | | 0.07±0.02 | NS | 0.17±0.02 |
| TGF-$\beta_1$ | 0.8±0.07 | NS | 0.8±0.1 | NS | 0.9±0.05 | NS | 0.7±0.08 |
| PDGF-$\alpha$ | 2.45±0.54 | NS | 2.09±0.62 | NS | 1.77±0.09 | NS | 1.68±0.13 |

Densitometric comparison of IL-2R, TGF-$\beta$, and PDGF-A bands with $\beta$-actin bands. No mRNA expression for IL-2R could be detected in the C + P and the A + P groups.

Table 3. IL-2R[alpha] (CD 25), TGF-[beta]$_1$, and PDGF-A mRNA synthesis 24 weeks after transplantationDensitometric comparison of IL-2R, TGF-[beta], and PDGF-A bands with [beta]-actin bands. No mRNA expression for IL-2R could be detected in the C + P and the A + P groups.
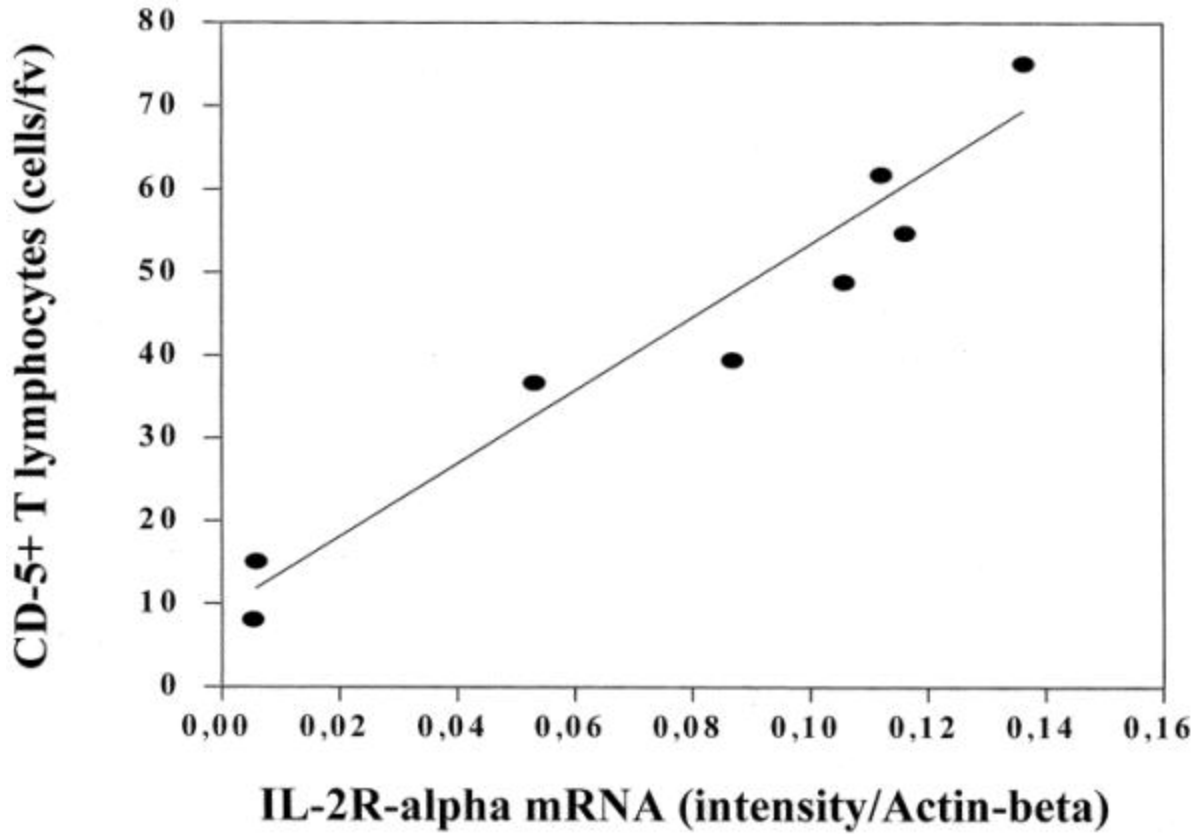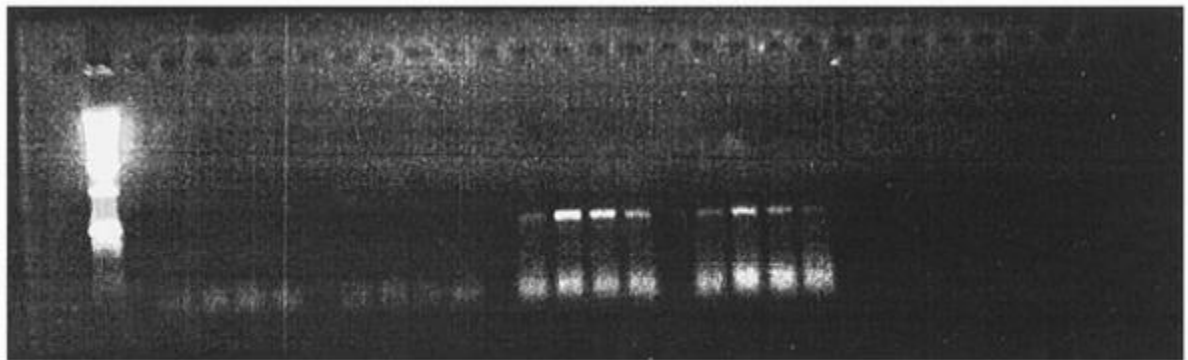
Figure 2. Correlation between CD5+ T lymphocyte count and IL-2R[alpha] mRNA expression (r=0.98) (P <0.001), 24 weeks after transplantation, in rats of the P and the control groups.



C+P          A+P          P          controls

Figure 3. IL-2R[alpha] mRNA expression in kidney allograft samples 24 weeks after transplantation by RT-

PCR. The PCR products are visualized on agarose gel stained with ethidium bromide.

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TGF-[beta]$_1$ mRNA synthesis was up-regulated to a small extent in all animals at week 24. There was no significant difference between the groups at this timepoint. The up-regulation of PDGF-A mRNA did not differ between the groups either (Fig. 3).

DISCUSSION

Presently, the ill-defined process of chronic rejection is the most important cause of allograft loss after transplantation. Although the introduction of cyclosporin A has improved short-term graft survival, the rate of attrition did not change over the long term (3). Current maintenance immunosuppressive therapy consist of cyclosporin in combination with prednisolone and azathioprine. Unfortunately these agents are associated with significant side effects resulting in posttransplant morbidity and mortality, including toxic injury of the allograft (23).

Immunohistochemical analysis of renal transplants undergoing chronic rejection revealed an infiltrate of both T lymphocytes and macrophages (18, 24, 25). Chronic rejection may be the result of a cell mediated immune reaction based on T lymphocyte recognition of the permanently present alloantigen (3, 4), recurrent but self-limiting episodes of acute rejection (26, 27) or alloantigen-independent events. Injury or local hemodynamic factors, such as hyperfiltration, may activate graft endothelial cells resulting in a non-alloantigen-dependent emigration of mononuclear cells into the allograft (17, 28). As lymphocytes are activated to some degree during extravasation, this suggests an involvement of activated T lymphocytes in chronic rejection.

Cyclosporin A blocks the synthesis of IL-2, although azathioprine inhibits T and B lymphocyte proliferation by interference with purine synthesis (3). Whether these therapeutic agents are beneficial in the process of chronic rejection, is under discussion (9). Some clinical studies suggested that a cyclosporin-based therapy is superior to azathioprine due to its more pronounced effects upon activated lymphocytes (10, 11), whereas other studies suggested a beneficial effect of a conversion from cyclosporin A to azathioprine due to the lack of azathioprine-related nephrotoxicity (12, 13). The long-term use of prednisolone has also been questioned, because of the serious side effects of long-term steroid treatment. Induction therapy with steroid-free cyclosporin regimen, as well as steroid-free maintenance therapy resulted in significantly more favorable 5-year graft and patient survival, than steroid-containing regimens according to reports of the European multicenter transplant study group (29). However as stated previously this may reflect a positive selection.

In culture of quiescent human T lymphocytes the IL-2 receptor [alpha]-chain (p55) (30) mRNA is not detectable (3, 31). The synthesis of IL-2R is highly up-regulated on T lymphocyte activation (3, 31). In our rat model, the synthesis of IL-2R was most pronounced in controls. Similar data have been presented by Krams et al. (30). who demonstrated the presence of IL-2R mRNA in human nephrectomy samples with pathological evidence for chronic rejection (30). In our study, both C+P and A+P significantly inhibited T lymphocyte infiltration, as well as the expression of IL-2R mRNA in parallel to a reduced pace of deterioration as indicated by functional parameters such as proteinuria or creatinine clearance.

In our study there was no difference in kidney function, morphology, or the expression of molecular markers between C+P and A+P therapy. Thus both strategies seem to be equally effective.

The long-term use of prednisolone in the treatment of renal allografts has been questioned (9, 14). In our study, prednisolone treatment alone had no effect on cellular infiltration, IL-2R mRNA expression, or allograft function, suggesting that this therapeutic agent might in fact be replaceable in the maintenance therapeutic regime of kidney transplant recipients. However, currently no recipient of graft is ordinarily treated with steroids alone. Furthermore we had no group with cyclosporin or azathioprine alone. Therefore this question needs further investigations.

Locally produced growth factors (TGF-[beta], and PDGF) may mediate tissue remodeling processes, such as chronic rejection of renal allografts (32). TGF-[beta]$_1$ plays a pivotal role in fibrogenesis and is an important mediator of fibrosis (33). In biopsies of chronically rejecting kidney allografts (24, 34), as well as in rat renal allografts with chronic rejection (35), the expression of TGF-[beta]$_1$ was elevated, suggesting that this factor may play an important role in chronic rejection (24, 35). Due to the potency of cyclosporin A to induce TGF-[beta]$_1$ in vitro and in vivo (36), it was hypothesized, that cyclosporin A may be harmful over the long term. In our study, mRNA synthesis of TGF-[beta]$_1$ was only minimally up-regulated in controls at week 24, and none of the treatment protocols altered TGF-[beta]$_1$ mRNA expression significantly.

TGF-[beta] is produced as an inactive molecule. Therefore, the active TGF-[beta] protein levels may not correlate with mRNA synthesis (35). Based on our immunohistological results, TGF-[beta] protein expression correlated with mRNA synthesis, although TGF-[beta] protein expression was significantly reduced in the A+P group despite similar RNA levels.

There is a growing body of evidence that in addition to TGF-[beta], PDGF synthesis is important in tissue remodeling such as progressive glomerulosclerosis (37, 38). Earlier studies suggested, that PDGF-AA might be more important than TGF-[beta] for the later phases of chronic rejection. In this study, PDGF-A mRNA

was more expressed, than TGF-$[beta]_1$, at week 16, however no differences were observed between the treated and the control groups, suggesting, that the effects of C+P and A+P treatment were not mediated through the inhibition of these growth factors in our study. There was no difference in the expression of PDGF-A or TGF-$[beta]_1$ mRNA between C+P and A+P either.

In summary, cyclosporin A or azathioprine may reduce the pace of chronic rejection of rat kidney allografts. Neither C+P nor A+P proved to be superior.

Acknowledgments.

REFERENCES

1. Isoniemi H, Nurminen M, Tikkanen MJ, et al. Risk factors predicting chronic rejection of renal allografts. Transplantation 1994; 57 (1):68. Bibliographic Links Library Holdings [Context Link]

2. Paul LC. Chronic renal transplant loss. Kidney Int 1995; 46:266. Library Holdings [Context Link]

3. In: Abbas AK, Lichtmann AH, Pober JS, eds. Cellular and molecular immunology. Philadelphia: WB Saunders Co, 1991: 226. [Context Link]

4. Orosz CG. Local cellular immunology of experimental transplant vascular sclerosis. Clin Transplant 1996; 10:100. Bibliographic Links Library Holdings [Context Link]

5. Hamar P, Müller V, Kohnle M, et al. Metabolic factors have a major impact on kidney allograft survival. Transplantation 1997; 64:1135. Ovid Full Text Bibliographic Links Library Holdings [Context Link]

6. Pabico RC, Yarger J, Byer B, Kremer M, McKenna BA, Cerilli GJ. Effects of cyclosporin A on chronic renal transplant rejection. Transplant Proc 1994; 26 (5):2577. Bibliographic Links Library Holdings [Context Link]

7. Hancock W, Whitley WD, Tullius SG, Heemann UW, et al. Cytokines, adhesion molecules, and the pathogenesis of chronic rejection of rat renal allografts. Transplantation 1993; 56:643. Bibliographic Links Library Holdings [Context Link]

8. Nadeu KC, Azuma H, Tilney NL. Sequential cytokine dynamics in chronic rejection of rat renal allografts: Roles for cytokines RANTES and MCP-1. Proc Natl Acad Sci USA 1995; 92:8729. [Context Link]

9. Opelz G. Effect of the maintenance immunosuppressive drug regimen on kidney transplant outcome. Transplantation 1994; 58:443. Bibliographic Links Library Holdings [Context Link]

10. Rosenblum ND, Harmon WE, Levy RH. Treatment of chronic renal allograft rejection with cyclosporin and prednisolone. Transplantation 1988; 45:232. Bibliographic Links Library Holdings [Context Link]

11. Abbud Filho M, Ramalho HJ, Barberato JB et al. Inhibition of chronic kidney allograft rejection by cyclosporin. Transplant Proc 1989; 21:1660. Bibliographic Links Library Holdings [Context Link]

12. Agarwal SK, Dash SC, Tiwari SC, et al. Results of conversion from triple-drug to double-drug therapy in living related renal transplantation. Transplantation 1995; 59:27. Bibliographic Links Library Holdings [Context Link]

13. Hollander AA, van Saase JL, Kootte AM, et al. Beneficial effects of conversion from cyclosporin to Azathioprine after kidney transplantation. Lancet 1995; 1: 8950, 610. Library Holdings [Context Link]

14. Opelz G. Influence of treatment with cyclosporin, azathioprine and steroids on chronic allograft failure. The collaborative transplant study. Kidney Int Suppl 1995; 52:s89. [Context Link]

15. Müller V, Hamar P, Szabó A, Vogelsang M, Philipp TH, Heemann U. In-vivo migration of lymphocytes in chronically rejecting rat kidney allografts. Transplant Int 1999; 12:145. Bibliographic Links Library Holdings [Context Link]

16. Isoniemi H, Krogerus L, Willebrand E, Taskinen E, Ahonen J, Häyry P. Histopathologic findings in well-functioning, long-term renal allografts. Kidney Int 1992; 41:155. [Context Link]

17. Rennke HG. Pathogenesis and significance of nonprimary focal and segmental glomerulosclerosis. Am J Kid Dis 1989; 13:443. Bibliographic Links Library Holdings [Context Link]

18. Heemann U, Azuma H, Hamar P, Schmid C, Tilney N, Philipp T. Mycophenolat mofetil inhibits lymphocyte binding and the upregulation of adhesion molecules in acute rejection of rat kidney allografts. Transplant Immun 1996; 4:77. [Context Link]

19. Chomczinsky P, Sacchi N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Ann Biochem 1987; 162:156. [Context Link]

20. Hartung J Lehr und Handbuch der angewandten Statistik. München: R Oldenburg Verlag, 1987, 612. [Context Link]

21. Li B, Sehajpal PK, Subramaniam A, et al. Inhibition of interleukin-2 receptor expression in normal human T-cells by cyclosporin. Demonstration at the mRNA, protein, and functional levels. Transplantation 1992; 53:146. [Context Link]

22. Li B, Sehajpal PK, Khanna A, et al. Differential regulation of Transforming growth factor beta and interleukin 2 genes in human T-cells: demonstration of usage of novel competitor DNA constructs in the quantitative polymerase chain reaction. J Exp Med 1991; 174:1259. Bibliographic Links Library Holdings [Context Link]

23. Kawamura T, Hullett DA, Suzuki Y, Bechstein WO, Allison AM, Sollinger HW. Enhancement of allograft survival by combination RS-61443 and DUP-785 therapy. Transplantation 1993; 55:691. Bibliographic Links Library Holdings [Context Link]

24. Heemann U, Tullius SG, Tamatami T, et al. Infiltration patterns of macrophages and lymphocytes in chronically rejecting rat kidney allografts. Transplant Int 1994; 7:349. Bibliographic Links Library Holdings [Context Link]

25. Frishberg Y, Meyers CM, Kelly CJ. Cyclosporine A regulates T cell-epithelial cell adhesion by altering LFA-1a and ICAM-1 expression. Kidney Int 1996; 50:45. Bibliographic Links Library Holdings [Context Link]

26. Lowry RP, Takeuchi T, Cremisi H, Konieczny B, Someren A. Chronic rejection of organ allografts may arise form injuries sustained in recurring foci of acute rejection that resolve spontaneously. Transplant Proc 1993; 25:2103. [Context Link]

27. Jiang H, Fujitsu T, Sakuma S, et al. Immunosuppressive Effects of FK 506 on Rat Renal Allograft Survival, in Comparison With Cyclosporine. Transplant Proc 1995; 27:367. Bibliographic Links Library Holdings [Context Link]

28. Schleimer K, Szabó A, Müller V, Hamar P, Heemann U, Eigler FW. Effect of the ACE inhibitor enalapril on development of chronic rejection after orthotopic rat kidney transplantation. Langenbecks Arch Chir Suppl Kongressbd 1997; 114:279. Library Holdings [Context Link]

29. Opelz G. Influence of treatment with cyclosporin, azathioprine and steroids on chronic allograft failure. The collaborative transplant study. Kidney Int Supp 1995; 52:s89. [Context Link]

30. Krams SM, Falco DA, Villanueva JC, et al. Cytokine and T cell receptor gene expression at the site of allograft rejection. Transplantation 1992; 53:151. Bibliographic Links Library Holdings [Context Link]

31. Li B, Sehajpal PK, Subramaniam A, et al. Inhibition of Interleukin-2 receptor expression in normal human T-cells by cyclosporine. Demonstration at the mRNA, protein, and functional levels. Transplantation 1992; 53:146. [Context Link]

32. Paul LC, Saito K, Davidoff A, Benediktsson H. Growth factor transcripts in rat renal transplants. Am J Kid Dis 1996; 28:441. Bibliographic Links Library Holdings [Context Link]

33. Border WA, Noble NA, Kettler M. TGF-[beta]: a cytokine mediator of glomerulosclerosis and a target for therapeutic intervention. Kidney Int 1995; 49:s59. [Context Link]

34. Horvath LZS, Friess H, Schilling M, et al. Altered expression of transforming growth factor-[beta]s in chronic renal rejection. Kidney Int 1996; 50:489. Bibliographic Links Library Holdings [Context Link]

35. Shihab FS, Yamamoto T, Nast C, et al. Transforming growth factor-[beta] and matrix protein expression in acute and chronic rejection of human renal allografts. JASN 1995; 6:286. [Context Link]

36. Wolf G, Thaiss F, Stahl RA. Cyclosporin stimulates expression of transforming growth factor-[beta] in renal cells. Transplantation 1995; 60:237. [Context Link]

37. Floege J, Alpers CE, Burns MW, et al. Glomerular cells, extracellular matrix accumulation, and the development of glomerulosclerosis in the remnant kidney model. Lab Invest 1992; 66:485. Bibliographic Links Library Holdings [Context Link]

38. Muchaneta Kubara EC, Sayed Ahmed N, El Nahas AM. Subtotal nephrectomy: a mosaic of growth factors. Nephrol Dial Transplant 1995; 10:320. Bibliographic Links Library Holdings [Context Link]

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