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ACE inhibition limits chronic injury of kidney transplant even with treatment started when lesions are established

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ACE inhibition limits chronic injury of kidney transplant even with treatment started when lesions are established.

Background. Inhibition of the renin-angiotensin system (RAS) prevents development of chronic allograft dysfunction in experimental animals. Whether this therapeutic approach is effective even if started when signs of allograft nephropathy are already manifested has not been investigated.

Methods. To address this issue, we studied the effect of a late treatment with the angiotensin-convertine enzyme (ACE) inhibitor trandolapril in the Fisher 344 to Lewis rat kidney transplant model. Seven months after transplant a renal biopsy was done for graft histology examination. Thereafter rats received either no treatment (allograft-none) or trandolapril until sacrifice at month 13.

Results. All animals were alive at the end of the study with the exception of a rat in the untreated group that died of renal insufficiency at day 292. Despite the fact that the grafts had already signs of structural injury and function impairment at the time treatment was stated, trandolapril completely restored renal function to baseline pretransplant values. Trandolapril also halted the progression of glomerular damage and suppressed intragraft T-lymphocyte infiltration and reduced the expression of the chemokine monocyte chemoattractant protein-1 (MCP-1). However, trandolapril had no direct effect on T cell function, since in vivo treatment did not modify recipient T-cell alloreactivity against donor antigens.

Conclusion. These findings provide the basis for a novel treatment intervention with RAS blockade that, together with pharmacologic inhibition of the immune response, could interrupt progression of chronic allograft dysfunction and injury.

Chronic allograft rejection is the most important cause of renal transplant loss [1]. This is a relentlessly progressive form of graft dysfunction, spanning years to decades and characterized morphologically by obliterative vasculopathy, interstitial fibrosis with variable degree of mononuclear cell infiltration, and, in the case of the kidney, glomerulosclerosis [2]. Although the functional and morphologic findings are well characterized, the pathophysiologic mechanisms leading to graft deterioration secondary to the evolution of this process are poorly understood.

Potential risk factors, other than immune events, include hypertension, inadequate functional nephron mass, drug toxicity, and de novo or recurrent progressive renal diseases [3, 4]. The functional and structural changes of chronic renal allograft nephropathy show similarities to those observed in other forms of chronic progressive renal disease in which inadequate functioning nephron mass has been considered the key event [5]. Indeed, transplantation of a single kidney theoretically supplies half the number of nephrons commonly available to health subjects. This implies an increased workload per nephron in order to maintain metabolic homeostasis [6]. Ischemic injury at transplantation, acute rejection, and chronic cyclosporine A (CsA) toxicity, contribute to further reducing the pool of functioning nephrons [5]. In experimental animals, an insufficient number of nephrons due to a reduction in renal mass (for acquired or innate reasons) triggers a self-perpetuating cycle of events, the hallmark of which is excessive urinary protein excretion followed by interstitial and glomerular inflammation and subsequent scarring [7]. Hemodynamic determinants of renal injury in this setting are enhanced intraglomerular pressure and flow, which are intimately involved in the development of lesions [8]. Glomerular hypertension enhances filtration of macromolecules across the capillary barrier, which are then largely reabsorbed by proximal tubular epithelial cells [7, 9]. Cell activation up-regulates genes for inflammatory and vasoactive proteins that, in the long run, also contribute to renal scarring [7].

Since no effective therapy exists so far for chronic allograft dysfunction, targeting above mechanisms may have implications for preventing progressive deterioration of renal function, which follows transplantation of single kidney. Rationale rests on findings that blockade of

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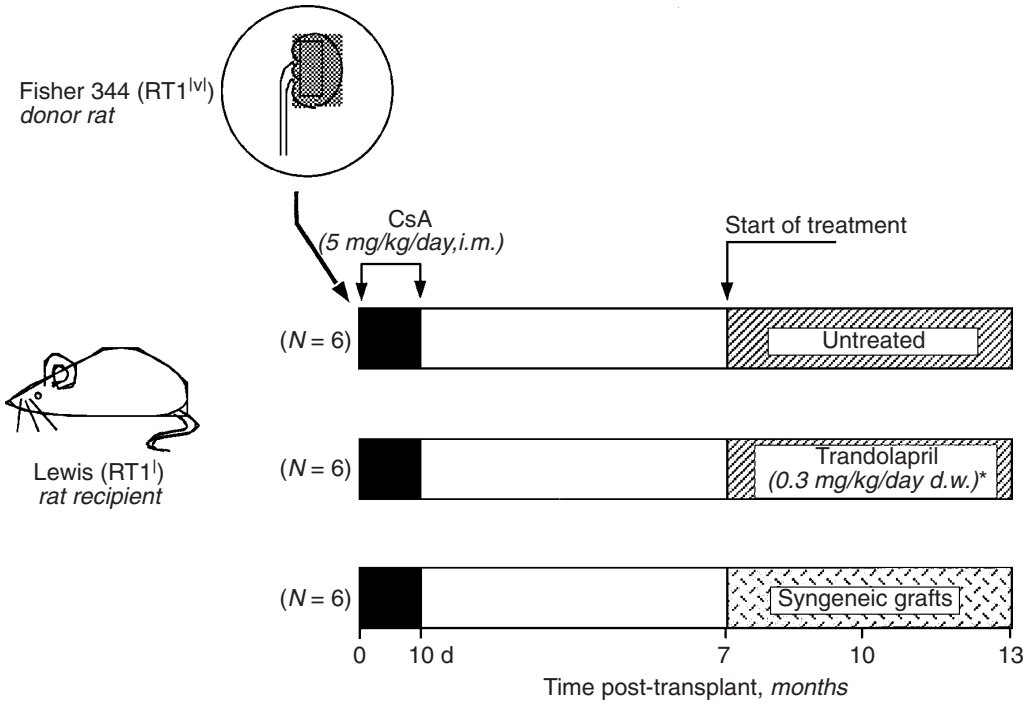

Fig. 1. Schematic representation of the experimental design. CsA is cyclosporine A.

the renin-angiotensin system (RAS) reduced the urinary protein excretion and protected against renal structural injury better than conventional antihypertensive therapy in nontransplant models of chronic renal disease, caused, however, by lower than normal nephron numbers [10], as well as in humans with proteinuric renal disease [4]. Studies showed already that RAS inhibition helped preventing chronic graft injury in the rat models [11–14]. The above, however, were designed as preventive interventions; therefore RAS inhibitors were started very early in the posttransplant course.

The question we wonder to address by the present study is whether such treatment is still capable of limiting disease progression or reverse lesions if started late enough in the course of the disease when signs of renal dysfunction are apparent. Answering to this might have major clinical implication. Therefore, we have used the Fisher 344 to Lewis rat kidney transplant model and initiated treatment with angiotensin-converting enzyme (ACE) inhibitor versus no treatment when rats had already proteinuria and renal function impairment. We also sough to investigate the mechanism(s) by which pharmacologic inhibition of the RAS afforded protection, if any, toward chronic allograft nephropathy.

METHODS

Animals

Inbred Fisher 344 (F344) and Lewis rats weighing 210 to 250 g were used in the experiments. Lewis rats (LEW RT1^{l}) acted as recipients and F344 (RT1^{lvl}) as donors. All animals were obtained from Charles River Italia (Calco, Italy), housed under standard conditions, and given rat food and water ad libitum. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n.116, G.U., Suppl 40, February 18, 1992; circolare No. 8, G.U., July 14, 1994) and international laws and policies (EEC Council Directive 86/609, OJL 358, December 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Kidney transplantation

Kidney transplantations were performed, as previously described [15]. Briefly, the left donor kidney was removed and positioned orthotopically into the recipients, whose renal vessels had been isolated and clamped, and left native kidney had been removed. End-to-end anastomosis of renal artery, vein, and ureter were performed using 10-O Prolene sutures (Ethicon, Inc, Somerville, NJ, USA). Total ischemia time was <30 minutes.

Experimental design

Two experimental groups of F344 to Lewis renal allograft recipients were studied (Fig. 1). An additional group 3 underwent Lewis to Lewis syngeneic kidney graft. All rats received CsA (5 mg/kg per day intramuscularly) (Novartis Farma, Milan, Italy) for the first 10 days after transplantation to prevent early acute rejection [16, 17]. After pre-transplant evaluation (baseline),

awake systolic blood pressure, serum creatinine concentration, and 24-hour urinary protein excretion were serially monitored up to the end of 7 months postsurgery. In addition, at this time point, a basal graft biopsy was performed in all groups of animals for pretreatment histologic analysis of kidney tissues. Thereafter, allografted animals were divided in the following groups, matched for urinary protein excretion and serum creatinine levels. Group 1 ($N = 6$) received no treatment and group 2 ($N = 6$) was given the ACE inhibitor trandolapril (0.3 mg/kg/day in the drinking water) (Abbott GmbH & Co., KG, Ludwigshafen, Germany). The dose of trandolapril was adjusted as needed to maintain systolic blood pressure within normal range. The additional group 3 animals ($N = 6$) were left untreated. All animals were followed up to the end of 13 months posttransplantation. Renal function, as serum creatinine, was monitored at monthly intervals. At the same time points, animals were place in individual metabolic cages for 24-hour urine collections and determination of urine output and protein excretion. Systolic blood pressure was measured serially in conscious rats by the tail-cuff method [18]. At the end of the study period, the animals were sacrificed and the kidney graft removed and processed for histology and immunohistology of graft cell infiltrate. Moreover, portions of renal allograft cortex were excised, snap-frozen in liquid nitrogen, and stored at –80°C for later analysis of gene expression of chemokines regulated upon activation, normal T cell expressed and secreted (RANTES) and macrophage chemoattractant protein-1 (MCP-1) and of transforming growth factor-β (TGF-β) by real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR).

To examine the effect of trandolapril on T-cell alloreactivity, two additional groups of naive Lewis ($RT1^l$) rats were studied. Group 4 ($N = 3$) was untreated controls and group 5 ($N = 3$) received trandolapril (0.3 mg/kg/day in the drinking water). Both groups were in vivo primed with intravenous infusion of allogeneic Brown-Norway (BN) ($RT1^n$) spleen cells (10×10^6). After 5 days, the animals were sacrificed, lymph nodes collected, and cell immunoreactivity was determined by mixed lymphocyte reaction (MLR) assay against donor BN or third-party Wistar Furth (WF) ($RT1^u$) splenocytes. For these experiments the Lewis-BN major histocompatibility complex (MHC) disparate combination was chosen since preliminary MLR experiments showed that the alloreactivity of either naïve or primed Lewis T cells against F344 splenocytes was very low.

Morphologic evaluation

Kidney specimens were fixed for 6 hours in Dubosq-Brazil and dehydrated in alcohol. After paraffin embedding, sections 3 μm in thickness (Ultrotome V) (LKB, Bromma, Sweden) were stained with Masson's trichrome, hematoxylin and eosin, and by the periodic acid-Schiff (PAS) techniques. The incidence and extension of glomerular and tubular structural lesions were estimated with slight modifications of previously described methods [16]. In particular, the frequency of focal and segmental sclerosis and hyalinosis was determined by examining all glomerular profiles contained within one or two coronal sections from each kidney and expressed as the percentage of the total number of glomeruli counted. A minimum of 80 glomeruli per kidney was evaluated for glomerulosclerosis. Tubular (atrophy, cast, and dilatation) and interstitial (fibrosis and inflammation) changes were graded on a scale of 0 to 4+: 0, no changes; 1+, changes affecting <25% on the sample; 2+, changes affecting 25% to 50% of the sample; 3+, changes affecting 50% to 75% of the sample; 4+, changes affecting 75% to 100% of the sample. All renal biopsies were analyzed by the same examiner, who was unaware of the nature of the experimental group.

Immunohistology

Mouse monoclonal antibodies were used for the detection of the following rat antigens: ED-1 antigen present in the rat monocytes and macrophages (Chemicon, Temecula, CA, USA), a rat MHC class II antigen monomorphic determinant (OX6) (Serotec, Oxford, UK), CD8 cell surface glycoprotein expressed by T cytotoxic suppressor cells (OX8) (Serotec), and CD4 cell surface glycoprotein, a 55 kD molecule expressed by helper T cells, thymocytes, and macrophage (W3/25) (Serotec). All antigens were analyzed by indirect immunofluorescence technique. The tissue fragments were frozen in liquid nitrogen, tissue sections (3 μm thick) were cut with a Mikrom 500 O cryostat (Walldorf, Germany) and fixed in acetone. The sections were blocked with phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA), incubated overnight at 4°C with the primary antibody (ED-1, 14 mg/mL; OX6, 5 μg/mL; OX8, 1:100; W3/25, 40 μg/mL), washed with PBS, and then incubated with Cy3-conjugated donkey anti-mouse IgG antibodies (affinity-purified, absorbed with rat IgG, 5 μm/mL in PBS) (Jackson Immuno-Research, West Grove, PA, USA) for 1 hour at room temperature. For the detection of intragraft IgG, direct immunofluorescence was performed using a fluorescein isothiocyanate (FITC)-conjugated goat antirat IgG antibody (Jackson Immunoresearch Lab, Baltimore, PA, USA) (30 μg/mL). For each marker, the number of cells was counted in at least 10 randomly selected high power microscope field (×400) for each animal.

Real-time quantitative RT-PCR

To analyze gene expression of RANTES and MPC-1 and TGF-β, RNA was treated with Dnase and reverse

transcribed to cDNA. Quantitative real-time PCR was performed on a TaqMan ABI Prism 5700 Sequence Detection System (PE Applied Biosystems, Monza, Italy) with SYBR Green PCR Core Reagents (Applied Biosystems), in combination with optimal primer concentrations. The amplification profile consisted of 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 minutes, then 60°C for 1 minute. All samples were run in triplicate. To assess the overall cDNA content, glyceraldheyde-3-phosphate dehydrogenase (GAPDH) served as housekeeping gene. The following oligonucleotide primers were used: rat RANTES (300 nmol/L), 5′-CCAACCTTGCAGTCGTCTTTG-3′ and 5′-TCTGGGTTGGCACACACTTG-3′; rat MCP-1 (300 nmol/L), 5′-CTGTCTCAGCCAGATGCAGTTAAT-3′ and 5′-GTGAATGAGTAGCAGCAGGTGAGT-3′; rat TGF-β (300 nmol/L), 5′-CCCTGCCCCTACATTTGGA-3′ and 5′-GGGCAAGGACCTTGCTGTACT-3′; and rat GAPDH (300 nmol/L), 5′-TCATCCCTGCATCCACTGGT-3′ and 5′-CTGGGATGACCTTGCCCAC-3′. Similar amplification efficiencies for targets and housekeeping genes were demonstrated by analyzing serial cDNA dilutions, showing an absolute value of the slope of log input cDNA amount versus Δ threshold cycle (Ct) (Ct target – Ct housekeeping gene) of <0.1. Thus, we used the ΔCt technique to calculate cDNA content in each sample. Melting temperature analysis evidenced no primer dimers or aspecific amplification products. Controls consisting of ddH_2O or RT-RNA were negative for targets and housekeeping.

Mixed lymphocyte reaction

Lymph node cells were isolated from responder Lewis rats by sieving and were cultured (1×10^6/well) with irradiated (4000 rad) splenocytes (1×10^6/well) from donor BN, third-party WF, or Lewis rats (control combination) for 5 days. Cells were then pulsed for 18 hours with 1 μCi [^{3}H]-thymidine and uptake of radioactivity was measured by liquid scintillation counting. The stimulation index (SI) was calculated as follows [19]:

$$SI = \frac{\text{cpm in allogeneic MLR}}{\text{cpm in control combination}}$$

Statistical analysis

Results are given as a mean ± SE. For functional parameters, the significance of differences between individual groups, after two-way analysis of variance (ANOVA), was established by using the Tukey-Cicchetti test for multiple comparisons. Estimates of renal injury from morphologic studies and immunohistochemical results were analyzed by the nonparametric Kruskal-Wallis test for multiple comparisons. Differences in real-time renal expression data between untreated and trandolapril-treated allograft recipients were analyzed by Student *t* test for unpaired data (two-tailed). Statistical significance was defined as $P < 0.05$.

Table 1. Body weight and systolic blood pressure in the three groups of transplanted rats

	Basal	Month 7	Month 9	Month 13
Body weight *g*				
Untreated	224 ± 8[a]	463 ± 13	489 ± 9	516 ± 25
Trandolapril	232 ± 5[a]	449 ± 15	485 ± 15	515 ± 16
Syngeneic	266 ± 13	456 ± 15	493 ± 12	520 ± 16
Systolic blood pressure *mm Hg*				
Untreated	118 ± 3	136 ± 4[a,b]	145 ± 3[b]	120 ± 6[c]
Trandolapril	118 ± 3	138 ± 3[b]	110 ± 7	105 ± 6[a]
Syngeneic	124 ± 3	121 ± 2	120 ± 4	130 ± 3

Data are mean ± SE.
[a] $P < 0.05$ vs. syngeneic; [b] $P < 0.05$ vs. basal; [c] $P < 0.05$ vs. trandolapril.

RESULTS

All rats grew at similar rates without significant differences in body weights between untreated, trandolapril, and syngeneic groups (Table 1).

Five out of six untreated recipients (group 1) survived up to the month 13 follow-up (mean survival 373 ± 16 days). In the trandolapril group (group 2), all six animals were alive at the end of the study period (mean survival 390 days). All six rats who underwent syngeneic kidney graft (group 3) survived up to the end of the follow-up.

As shown in Table 1, at 7 months after transplant (before starting any treatment) allografted rats from groups 1 and 2 were slightly hypertensive ($P < 0.05$ vs. baseline). In untreated rats, systolic blood pressure remained elevated until month 9 posttransplant, but thereafter spontaneously declined toward normal baseline values. In group 2 rats, who started trandolapril treatment at month 7 postsurgery, systolic blood pressure declined soon after beginning antihypertensive therapy, and remained well controlled throughout the follow-up (Table 1). In rats with syngeneic kidney grafts, systolic blood pressure did not change significantly during the entire study period.

Graft function

Figure 2 shows the time course of serum creatinine concentration as an index of renal function in animals from individual groups that were alive at given time points after transplant. At month 7 postsurgery, animals with kidney allografts had serum creatinine concentrations significantly higher ($P < 0.05$) than baseline pretransplant values or rats of group 3 with syngeneic grafts. In untreated animals of group 1, a further progressive increase in serum creatinine was found during the following the study period. Trandolapril reduced serum creatinine levels to the extent that, after 2 months of treatment, normal values (0.54 ± 0.03 mg/dL), comparable to pretransplant

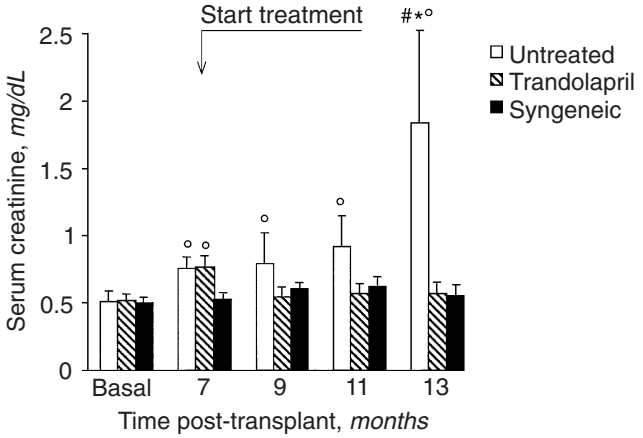

Fig. 2. Time course of serum creatinine in the three groups of transplanted rats. Untreated is untreated allograft recipients. Values are the mean ± SE. °$P < 0.05$ vs. basal and syngeneic; #$P < 0.01$ vs. trandolapril and syngeneic; *$P < 0.01$ vs. 7 months.

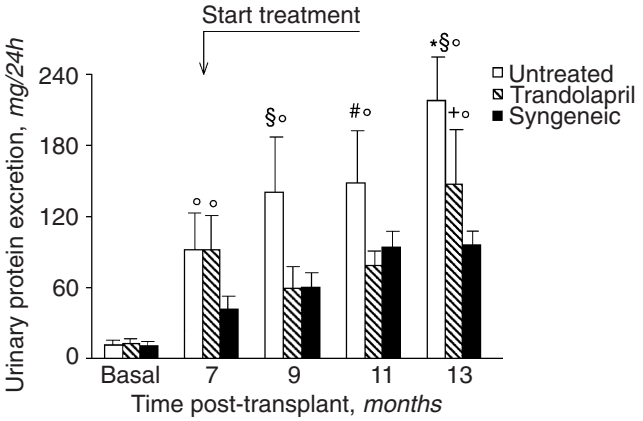

Fig. 3. 24-hour urinary protein excretion of the different experimental groups as a function of time after transplant. Untreated is untreated allograft recipients. Values are mean ± SE. °$P < 0.05$ vs. basal; #$P < 0.05$ vs. trandolapril and syngeneic; *$P < 0.01$, $^{+}P < 0.05$ vs. 7 months; §$P < 0.05$ vs. syngeneic.

basal values (0.52 ± 0.01 mg/dL), were achieved. At this time point, serum creatinine in this group was similar to that of syngeneic transplanted rats (0.54 ± 0.04 mg/dL). At the end of the study, trandolapril-treated rats showed normal graft function, with serum creatinine values significantly ($P < 0.01$) lower than untreated group 1.

As depicted in Figure 3, urinary proteins excretion was significantly higher ($P < 0.05$ vs. baseline) at month 7 posttransplant in both groups 1 and 2 who had kidney allografts than at pretransplant baseline. At the same time, protein excretion in syngenic graft recipients was numerically lower than in the allograft groups. Progressive increase in urinary proteins was observed over the following 6 months in untreated rats. Chronic inhibition of the RAS with trandolapril in group 2 initially not only prevented, but also reversed progressive urinary protein excretion during the next 4 months, reaching values comparable to those of syngeneic grafted rats ($P < 0.05$ vs. group 1 at month 11). Thereafter, however, proteinuria started again to increase, so that at the end of the study period mean value was significantly higher than at pretreatment month 7 ($P < 0.05$). Nevertheless, in this group, the urinary protein excretion was numerically lower than in untreated rats of group 1, but higher than in syngeneic graft rats. An age-related slowly progressive increase in protein excretion rate was found in animals with kidney syngeneic transplant.

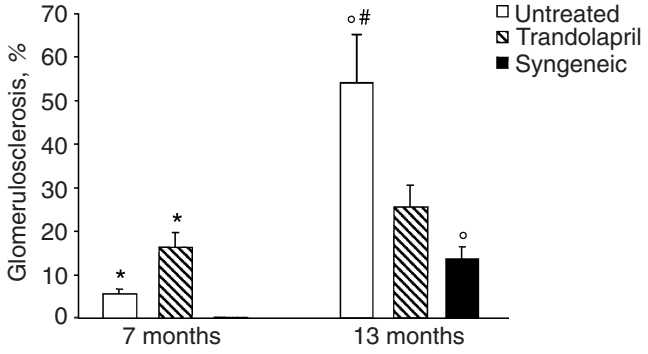

Fig. 4. Glomerular injury in the three groups of transplanted rats at 7 months (before start of treatment) and at the end of the 13-month follow-up period. Untreated is untreated allograft recipients. Values are mean ± SE. °$P < 0.05$ vs. 7 months; #$P < 0.01$ vs. trandolapril and syngeneic; *$P < 0.05$ vs. syngeneic.

Table 2. Tubulointerstitial injury in the three groups of transplanted rats

	Untreated	Trandolapril	Syngeneic
Tubulointerstitial injury (score)			
Month 7	0.41 ± 0.2[a]	0.58 ± 0.3[a]	0.20 ± 0
Month 13	1.90 ± 0.4[a,b]	1.60 ± 0.5	0.87 ± 0.1[b]

Data are mean ± SE
[a]$P < 0.05$ vs. syngeneic; [b]$P < 0.05$ vs. 7 months

Graft morphology

Histology findings from graft biopsies performed at months 7 and 13 posttransplant are reported in Figure 4 and Table 2. Morphologic evaluation on month 7 pretreatment biopsies showed focal and segmental glomerulosclerosis affecting 5.7% ± 2.6% and 16.5% ± 4.9% of glomeruli of group 1 and 2 allografts ($P < 0.05$ vs. syngeneic grafts at 7 months), whereas glomerular injury was negligible in syngeneic grafts (Fig. 4). At month 13, sections of allografts from untreated rats showed further increase in glomerular injury involving 54.0% ± 11.5% of glomeruli ($P < 0.05$ vs. 7 months). Trandolapril halted the progression of graft glomerular lesions, so that 25.4% ± 10.2% of glomeruli were affected, a value only numerically higher than in the same group 2 at pretreatment month 7, but significantly ($P < 0.01$) lower than that in untreated rats of group 1 (Fig. 4). In keeping with

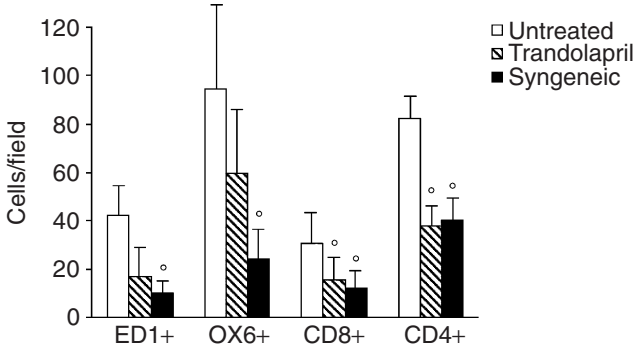

Fig. 5. Intragraft immunostaining of ED-1$^+$ macrophages, OX6+ [major histocompatibility complex class II (MHC II$^+$)] cells, CD8$^+$, and CD4$^+$ lymphocytes in the three groups of transplanted rats at the end of the 13-month follow-up. Untreated is untreated allograft recipients. Values are mean ± SE. $^\circ P < 0.05$ vs. control.

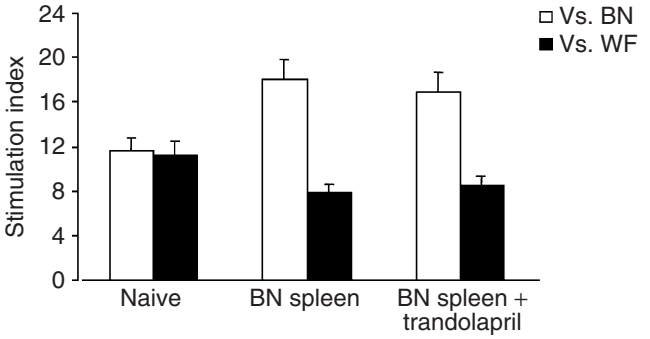

Fig. 6. Effect of in vivo administration of trandolapril on T-cell alloreactive response. Lewis rats received a pulse of allogenic Brown-Norway (BN) splenocytes (10×10^6 intravenously.) and were either left untreated (BN spleen) or were treated with trandolapril (BN spleen + trandolapril). Five days later, animals were sacrificed and lymph node cells were isolated. The lymphocyte proliferative response (stimulation index) against donor BN or third-party Wistar Furth (WF) stimulators was assessed in mixed lymphocyte reaction (MLR) in vitro. Spleen is splenocytes.

changes of proteinuria, glomerular injury increased with time in syngeneic grafts reaching at 13 months statistical significance ($P < 0.05$) as compared to 7 months. Tubulointerstitial injury in untreated rats of group 1, already present at month 7 postsurgery, was markedly and significantly higher at the end of the study period (Table 2) ($P < 0.05$ vs. 7 months). Trandolapril partially limited the increase of tubulointerstitial injury, whose mean score value at month 13 posttransplant was numerically lower than in untreated rats, but higher than in syngeneic grafts (Table 2).

Immunohistology

A detailed immunohistology evaluation of allografted and syngeneic animals studied at the end of the 13-month follow-up was undertaken (Fig. 5). In kidney from trandolapril-treated rats, interstitial infiltration of CD4$^+$ and CD8$^+$ T cells was significantly ($P < 0.05$) lower than in untreated rats of group 1. Similar results were found with ED-1$^+$ monocyte/macrophages ($P < 0.05$ vs. untreated) and MHC class II cells (OX6$^+$) that were numerically lower in trandolapril-treated than in untreated animals. No significant difference in the interstitial accumulation of infiltrating cells between trandolapril and syngeneic groups was found for each of the cell types considered. Moreover, in trandolapril-treated rats, mean score of IgG deposition at the end of the study period was numerically but not significantly lower than in untreated rats of group 1 (glomeruli, trandolapril 0.58 ± 0.66 and untreated 1.50 ± 1.00; cortical tubuli, trandolapril 1.58 ± 1.43 and untreated, 2.40 ± 0.89; and medullary tubuli, trandolapril 1.00 ± 1.55 and untreated, 1.20 ± 1.30).

Intragraft expression studies

Real time RT-PCR studies on allograft tissue at the end of the 13-month follow-up revealed a beneficial effect of trandolapril on intragraft MCP-1 expression that was significantly lower than in untreated rats of group 1 (trandolapril, MCP-1/GAPDH, 0.0073 ± 0.0035 vs. untreated 0.023 ± 0.07, $P < 0.05$). At variance, no significant difference in RANTES and TGF-β expression was found in trandolapril-treated and in untreated rats (RANTES/GAPDH, 0.030 ± 0.024 vs. 0.032 ± 0.033; TGF-β/GAPDH, 0.033 ± 0.005 vs. 0.032 ± 0.012).

T-cell alloreactivity by MLR

In additional nontransplanted Lewis rats, the effect of short-course trandolapril on T-cell immune response was also evaluated (Fig. 6). Lymphocytes from naive Lewis rats normally proliferated in vitro when stimulated with either BN or WF splenocytes. T cells from Lewis rats previously pulsed with BN allogeneic splenocytes further proliferated when cultured in vitro with donor BN but not third-party WF stimulator splenocytes. In vivo treatment with trandolapril had no effect on Lewis T cell alloreactivity against donor BN or third-party WF alloantigens.

DISCUSSION

Results of the present study show that a late treatment with the ACE inhibitor trandolapril fully restored normal graft function, limited progressive proteinuria, and stabilized glomerulosclerosis changes in a rat kidney transplant model with chronic renal dysfunction and overt proteinuria. These data indicate that, once initiated, chronic rejection is not necessarily an inevitable process but can be interrupted by the RAS blockade. This is the first experimental attempt of inhibiting RAS as therapeutic strategy to enhance graft longevity and preserve adequate renal allograft function in a setting

of graft dysfunction and overt chronic proteinuria. The experiments were performed in the Fisher to Lewis rat model of renal transplantation that is considered to be a useful model of chronic allograft nephropathy [20]. The progressive changes occurring mirror most, although not all, the changes experienced by patients with chronic allograft nephropathy who themselves manifest proteinuria of varying degree, in conjunction with progressive diffuse glomerulosclerosis, interstitial lesions, and vascular obliteration [21].

Few reports are available on the effects of late inhibition of the RAS on renal function and structure in nontransplanted experimental rat models of renal disease progression [22–27]. ACE inhibitors given late during the animal's life, when animals were already heavily proteinuric, decreased blood pressure and proteinuria, and stopped the disease from progressing in Munich Wistar Fromter (MWF) rats [26], genetically programmed to develop proteinuria and progressive renal damage with age. Moreover, sclerosis was remodeled in aging rats by inhibiting the RAS with an angiotensin II (Ang II) receptor antagonist given at high doses for 6 months [27]. More recently, combination of an ACE inhibitor and an Ang II receptor antagonist, in order to maximize the Ang II blockade, improved glomerular ultrafiltration coefficient (K_f), fully eradicated proteinuria, and partially regressed the early glomerulosclerotic lesions and tubulointerstitial injury in MWF rats [28]. Thus, while progressive deterioration of renal function was previously considered an inexorable process, occasional observations in humans and subsequent, more systematic studies in both humans and experimental animals have indicated collectively that improvement of renal function, remission of proteinuria, and even regression of renal structural injury may actually occur [29]. Here, we have documented that there may also be a window of opportunity for chronic allograft nephropathy where specific interventions, such as RAS blockade, may halt or even regress the pace of disease progression. In the present study, graft dysfunction was rapidly normalized by trandolapril, and serum creatinine concentration remained thereafter stable within the normal range. Together, normalized graft function, remarkably reduced urinary protein excretion, stable glomerular structural lesions, and limited tubulointerstitial changes, indicate that late ACE inhibition allowed the interruption of the progression of chronic allograft nephropathy.

After the initial marked reduction in urinary protein excretion, while still on chronic ACE inhibitor treatment, proteinuria started again to slowly increase. An explanation could be that the dose of ACE inhibitor used was not high enough to completely block the RAS eventually resulting in the slow escape from the ACE inhibition and its antiproteinuric effect. An alternative pathway to graft injury should be also taken into consideration in the relapse of proteinuria we found in trandolapril-treated rats. Recent evidence emphasized contributions of alloantigen-dependent factors in the pathogenesis of chronic renal allograft failure [4], which add to the potential deleterious effect of Ang II. The above involve intragraft T-cell and macrophage activation [30–32]. In particular, it has been suggested that continuous T-cell recognition of alloantigens, presumably via the indirect pathway, and full cell activation through costimulatory signals are events that may play an important role in the progression of chronic rejection [31]. Target interventions of blocking T-cell costimulatory activation could be of value. This possibility is supported by previous findings in the same experimental rat model of chronic kidney allograft rejection that late blockade of T-cell costimulation with the fusion protein CTLA4Ig transiently reduced urinary protein excretion [31]. Since in our animals trandolapril was the only single chronic treatment used, the ongoing immune response may have been left unabated, accounting for the alloantigen-dependent and cell-mediated contribution to proteinuria. Finding that trandolapril, given in vivo to nontransplanted rats pulsed with allogeneic splenocytes, did not affect ex vivo T-cell alloreactivity against donor or third-party alloantigens in the MLR assay seems to rule out the possibility that Ang II inhibition in this model could exert immunomodulatory properties [33].

Among the factors associated with tissue injury in progressive renal disease, other than glomerular hypertension and proteinuria, are infiltrating mononuclear cells, macrophages and T cells, which become activated and participate in parenchyma scarring by synthesizing and secreting fibrogenic cytokines, which stimulate interstitial fibroblast proliferation and matrix accumulation [7, 34, 35]. The key role of infiltrating cells in inducing interstitial injury leading to further inflammation and fibrosis has been documented extensively in the last few years [7, 36]. Furthermore, therapeutic interventions that dampen the interstitial inflammatory response preserve renal function from progressive deterioration [7]. In this context, the interstitial inflammatory reaction in biopsy specimens from trandolapril-treated rats at the end of the 13-month follow-up was markedly lower than in untreated allografts. It is tempting to speculate that limiting protein excretion rate and tissue inflammatory response by pharmacologic inhibition of Ang II synthesis may have synergistically contributed to preserve graft function within the normal range. Interestingly, recent evidence also suggests that Ang II may promote the synthesis of a variety of cell-adhesion molecules and cytokines possibly via reactive oxygen intermediates and activation of the transcription factor nuclear factor-kB (NF-kB) [37–39]. Molecules with NF-kB-responsive elements include intercellular adhesion molecule-1 (ICAM-1) and MCP-1. Coordinated expression of these latter factors might be

expected to promote local macrophage and lymphocyte recruitment in the allograft. Our data show that inhibition of RAS also attenuated the intragraft cortical expression of the potent macrophage and lymphocyte chemokine, MCP-1, which may ultimately have been related to the beneficial effect of the treatment on the interstitial inflammatory cell infiltration. These findings may be considered consistent with suppression of leukocyte-associated cytokine and leukocyte-chemoattractant expression by Ang II receptor blockade in Fisher to Lewis rat kidney allografts, when treatment was started very early postsurgery [14].

CONCLUSION

Our data indicate that inhibition of Ang II synthesis induces a complete normalization of graft function and some degree of remission of proteinuria, and stabilized kidney structural changes even if started late in the course of the disease, when lesions are already established. These effects are associated with reduction in allograft cell infiltration and attenuated expression of molecules involved in macrophage and lymphocyte recruitment. Translating these considerations into clinical transplantation, these findings provide the basis for a novel treatment intervention with RAS blockade that, together with pharmacologic inhibition of the immune response, could interrupt progression of chronic allograft dysfunction and injury. This eventually would result in prolongation of graft survival and reduction of the rate of kidney allograft loss.

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