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Endothelin-1, endothelin-3 and their receptors (endothelin$_A$ and endothelin$_B$) in chronic renal transplant rejection in rats[1]

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Abstract Endothelins (ET) are a family of vasoactive peptides that play an important role in several disorders affecting kidneys. In this study we investigated the expressions of ET-1, ET-3, and their receptors, ET_A and ET_B, in a rat chronic renal transplant rejection model. Renal allografts were performed (F344 → Lewis) with bilateral nephrectomy in recipients. For isograft control, lewis → lewis transplantations were performed. All recipients were sacrificed 140 days after transplantation and the grafts were analyzed histologically. ET-1 and ET-3 protein expression in grafts was measured by immunohistochemistry and ELISA. Semiquantitative RT-PCR methods were used for mRNA levels of ET-1, ET-3, ET_A and ET_B. No evidence of chronical rejection was manifested in isografts. The allografted rats showed proteinuria and increased serum creatinine levels. Histologically, renal allografts showed atrophy and sclerosis of the glomeruli, cortical scarring and vascular intimal thickening. Immunohistochemically, ET-1 and ET-3 were localized in the convoluted tubules, collecting ducts, endothelium and smooth muscle cells of the large blood vessels. Significantly increased staining for ET-1 and ET-3 were found in allografts compared to isografts. Simultaneously, ELISA for ET-1 and ET-3 showed elevated protein concentrations in allografts compared to isografts. Allografts showed significantly increased ET-1- and ET-3 mRNA compared to isografts. On the other hand, a significant down regulation of the ET_A mRNA was noted, and the ET_B mRNA remained unchanged. The data from the present study suggest that alteration of ET system may be of importance in the pathogenesis of chronic renal transplant rejection.

Key words Endothelin-1 · Endothelin-3 · Chronic renal rejection · Rat renal transplantation

Abbreviations *ET* Endothelin · *CyA* Cyclosporine A · *LEW* Lewis rat · *F344* Fisher 344 rat · *IFN* Interferon

Introduction

Due to its profound medical, financial and emotional consequences chronic rejection is the greatest impediment to successful long-term graft survival. The main histological feature of chronic rejection is an obliterative vasculopathy leading to development of glomerulosclerosis and progressive end-stage organ failure. Several factors including immune response, perfusion failure, hemodynamic alteration, endothelial damage, and inflammatory reaction may all in part be responsible for its pathogenesis [27]. Many of these pathogenetic processes may alter the production of vasoactive peptides in the allografts. Vasoactive molecules like endothelin

(ET) may play an important role in hypertension, ischemic-reperfusion injury, and possibly in pathological changes associated with inappropriate vascular-cell proliferation [31]. However, the role of ET in transplant rejection has not been fully defined.

Four isoforms of ET (ET-1, ET-2, ET-3, and vasoactive intestinal contractor) have been found in mammals [28]. The ETs are a family of 21-amino acid peptides with potent vasoactive properties [2]. In addition, ETs are involved in morphogenesis, smooth muscle contraction and steroidogenesis [11, 14, 22]. The biological effects of ETs results from binding to several receptor subtypes, two of which (ET_A and ET_B) have been cloned from mammalian cells [24].The differences in tissue-specific expression of the two receptors contribute to the different actions of the endothelins [2].

In the kidney, both ET-1 and ET-3 are present, although ET-1 is probably of greater importance [12, 24]. The physiological roles of ETs include vasoregulation, mitogenesis, natriuresis and diuresis [12, 24]. It was demonstrated that ET-1 elevates c-fos, c-jun and c-myc expression and extracellular matrix protein synthesis [16, 17]. Many of the studies of the role of ET in kidneys have concentrated on ET-1, very few studies of ET-3 have been reported. Circumstantial evidence exists for the potential pathophysiological role of ET in several renal abnormalities such as ischemia-induced acute renal failure, cyclosporine A- (CyA) and FK506 nephrotoxicity, radiocontrast nephrotoxicity, hepatorenal syndrome, and polycystic kidney disease [7, 13, 24, 29, 32].

It is known that elevated plasma levels of ET, found in patients with renal insufficiency, return to baseline after successful renal transplantation [31]. Raised ET-1 blood levels were detected during vascular rejection in association with reduced ET-1 immunostaining in the endothelium [33]. In cellular rejection, where endothelial cells are intact, no significant elevation of plasma ET-1 was observed [32]. However, blood levels do not reflect the true biological significance of ET well since these peptides are rapidly cleared and only very high concentrations are detected. Significant tissue specific upregulation of ET-1 may occur without any detectable alteration in plasma ET levels [30]. Local upregulation of ET-1 gene and peptide was shown in a model of chronic cardiac transplant rejection [30]. In kidneys, the degree of glomerulosclerosis correlated with an increased ET-1 expression in a non-transplant rat nephrectomy model [3, 20]. Increased ET may cause smooth muscle cell and mesangial proliferation and increase matrix protein synthesis leading to graft arteriosclerosis and glomerulosclerosis [24]. However, detailed long-term studies of ETs and their receptors after kidney transplantation are lacking. For this reason, the current study was undertaken to evaluate the role of ETs and their receptors in the pathogenesis of chronic renal transplant rejection.

Materials and methods

Animals and treatments

The experiments were carried out in a well characterized model of chronic transplant rejection [8]. The two strains of rats: Lewis (LEW) and Fisher 344 (F344), were obtained from Jackson Laboratories (Bar Harbor, Maine, USA) and housed at the animal care facility, University of Western Ontario, London, Ontario in accordance with guidelines established by the Canadian Council on Animal Care (1984). Only male rats weighing 250–300 g were used for transplantation. Renal transplantations were performed using end-to-end anastomosis between donor renal vessels and recipient abdominal aorta and inferior vena cava. Ureteric reconstruction was established by anastomosis between donor ureter and recipient bladder [8]. The native kidneys were removed during the grafting. Two groups ($n = 10$ for each group) of rats were investigated: the isogenic LEW to LEW combination controlled the success of the operation, and the allogenic F344 to LEW combination represented the rejection model. All LEW recipients with F344 allografts were treated with a subtherapeutic dose of CyA at 1.5 mg/kg per day for 10 days to prevent an initial acute rejection episode.

Tissue collection

In a pilot study, we established that after approximately 140 days of follow-up, the animals develop histological changes consistent with chronic rejection. This is in keeping with previous studies in this model [1]. Therefore, 140 days after renal transplantation, the rats were sacrificed and the kidneys removed. Three quarters of each transplanted kidney was snap frozen in liquid nitrogen and was stored at –80 °C until analysis. The remaining tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. The serum and urine of each individual rat was collected just before the removal of the graft. Proteinuria were assessed using test strips (URISCAN, Yeong Dong Co. Seoul, Korea).

Estimation of the creatinine levels in serum and urine samples

Creatinine assays were performed using a commercially available test kit following instructions provided by the manufacturer (Sigma Chemical Company, St. Louis, MO, USA)

Histological assessment

Five μm thick sections were cut consecutively from the paraffin embedded tissue blocks, mounted onto glass slides and used for staining. Each specimen was stained with hematoxylin and eosin, periodic acid schiff stain, and trichrome stain. Each section was assessed for rejection and was given a score of rejection. The investigator was unaware of the source of the specimen. All tissues were analyzed independently by at least two investigators.

Immunohistochemical localization of endothelins

Immunohistochemical localization of ET-1 and ET-3 were performed as described earlier by our group [6]. Briefly, sections were deparaffinized and rehydrated followed by incubation with 3% H_2O_2 with methanol for 30 min to remove the endogenous peroxidase activity. Sections were further incubated with 10% normal goat serum for 1 h, followed by incubation with polyclonal rabbit

antiserum (1:400, Peninsula Laboratories, Inc., Belmont, Calif., USA) at 4 °C overnight. The specificity of the antibody was established [6]. The sections were then washed in Tris-HCl buffer (0.05 M, pH 7.6) and sequentially incubated with 1) biotinylated goat anti rabbit IgG for 45 min at room temperature, and 2) peroxidase-anti-peroxidase complex for 1 h at room temperature. The sections were stained by immersing slides in 0.05% 3,3'diaminobenzidine tetrahydrochloride in the presence of 0.33% hydrogen peroxide. All slides were counterstained with hematoxylin and 0.3% ammonia water, dehydrated and mounted.

Enzyme immunoassay for ET-1 and ET-3

ETs were extracted from rat tissues according to the method of Matsumoto et al. [15]. Briefly, approximately 0.5 g wet weight of kidney homogenized with a polytron homogenizer for 60 s in 4 volumes of 1 M acetic acid containing 10-μg/ml pepstatin (Sigma Chemical Company, St. Louis, Mo., USA) and boiled for 10 min. After centrifuging at 25,000 × *g* for 30 min at 4 °C, the supernatant was collected and was applied to a Sep-pak C18 cartridge (Waters, Mass., USA). The adsorbed peptides were eluted and lyophilized for further assay. Competitive enzyme immunoassays for ET-1 and ET-3 were conducted according to the manufacturer's guidance (Peninsula Laboratories Inc., Belmont, Calif., USA). Equal amount of samples or standards (50 μl each) was dispensed into the immunoplate. Then, 25 μl of primary antiserum and 25 μl of biotinylated peptide solution were dispensed into each well. The immunoplate was incubated for 2 h at room temperature. After that, the plate was washed with assay buffer and then incubated with 100 μl of streptavidin-horse radish peroxidase (SA-HRP) solution (1:2000) for another hour. After washing away excess SA-HRP, TMB (3, 3'5, 5'Tetramethyl Benzidine Dihydrochloride) was added as the substrate for HRP. Adding 100 μl of 2 N HCL into each well terminated the reaction. The absorbency at 450 nm of the immunoplate was recorded.

RNA isolation and quantitative RT/PCR

TRIZOL reagent (Canadian Life Technologies Inc. Burlington, Ontario, Canada) was used to isolate RNA from the kidney. Following homogenization, RNA was extracted with chloroform, recovered from the aqueous phase by precipitation with isopropyl alcohol and suspended in DEPC-water. First strand cDNA synthesis was performed using the Superscript-II system (Canadian Life Technologies Inc. Burlington, Ontario, Canada). The resulting RT products were stored at –20 °C.

The polymerase chain reaction was carried out using previously described methods by our laboratory [5]. The rat GAPDH mRNA was used as a housekeeping gene for the PCR reaction. In order to define the optimal amplification conditions, a series of pilot studies was performed using various amounts of RT products from 0.25 to 2 μg RNA and 20 to 40 cycles of PCR amplification. A set of representative data showing the linear amplification of the ETs is in Fig. 3. The following conditions were established as standard for the PCR reactions: RT products from 1 μg RNA, 2.5 U Taq polymerase, 1 × PCR buffer, 1.5 mM $MgCl_2$, 250 μM dNTP mix, 1 μM of each primer. Thirty cycles of amplification were chosen in a final volume of 50 μl for ET-1, ET-3 and GAPDH. Forty cycles were used for amplification for ET_A and ET_B in a final volume of 30 μl. The amplification was carried out as follows: the initial cycle for 3 min at 94 °C for denaturation, 1 min at 54 °C for annealing, and 3 min at 72 °C for extension. Subsequent cycles of PCR were performed under the following conditions: denaturation, 45 s at 94 °C; annealing, 45 s at 54 °C; and extension, 1 min at 72 °C and 7 min for final extension.

The amplification products were analyzed on a 2.5% agarose gel in 1 × TAE buffer. 10 μl of each PCR product was loaded in each lane and electrophoresed at 100 volts for 90 min and visualized with UV light following ethidium bromide staining. Subsequently, the PCR products were transferred from the gel onto nylon membranes. Hybridizations were performed with biotinylated amplification product-specific oligoprobes [5]. The detection was carried out using a NBT/BCIP system.

No amplification products were found when dH_2O was used in the RT-PCR reaction for each set of primers. Quantitation of the PCR products was carried out by serial dilution slot-blot hybridization and densitometry of the products from the upstream of amplification onto nylon membranes using the same oligoprobes as previously described [8]. The membranes were analyzed by a Hewlett-Packard 4 C scanner and using Mocha software (Jandel Scientific, California, USA). The densitometric values were calculated as arbitrary units per μg of total RNA.

Statistical analysis

Statistical evaluation of data was performed by the unpaired student t-test using the Graphpad Instat statistical packages (GraphPad Software, San Diego, Calif., USA). Statistical significance was chosen for $P < 0.05$. All results were presented as mean ± standard error (SE).

Results

Most (90%) of the allograft recipients and all of the isografted animals survived for 140 days. Treatment with the conventional immunosuppressive agent CyA for the first 10 days in allografted rats prevented early loss of renal allografts. Such grafts, however, develop functional and morphological changes of chronic rejection. Serum creatinine levels were significantly raised (2.03 + 0.6 mg/dl for isografts vs. 4.42 + 2.7 mg/dl for allografts, $P < 0.05$), while urine creatinine levels were significantly decreased (100.2 + 46.4 mg/dl for isografts vs. 44.1 + 24.3 mg/dl for allografts, $P < 0.05$) in allografted animals compared to isografts, suggesting an impaired renal function of the allografts. Allografted animals showed presence of proteinuria in the range of 20 g/l or greater compared to less than 1 g/l in the isografts.

Characteristic morphological chronical rejection in the rat was observed in most (87.5%) allografted rats (Table 1). Fifty percent of the allografts showed glomerular loss and glomerulosclerosis. Hyelinization and vascular intimal thickening was present in 87.5% of the cases. More than 60% of allografts showed tubular atrophy and tubulitis. The majority (87.5%) of allografts contained interstitial monocyte infiltration and fibrosis that were prominent in cortical region.

On the other hand, isografted kidneys were devoid of morphologic features that were characteristic for chronic rejection (Table 1). Only minor changes, such as tubu-

Table 1 Histology in rat transplanted kidneys

	ISOGRAFT ($n = 9$)	ALLOGRAFT ($n = 8$)
Glomerular		
Glomerular loss	0/9 (0%)	4/8 (50%)
Atrophy/sclerosis	0/9 (0%)	5/8 (62.5%)
Vascular		
Endothelitis	0/9 (0%)	2/8 (25%)
Hyalinization/intimal Thickening	0/9 (0%)	7/8 (87.5%)
Tubular		
Atrophy	2/9 (22%)	5/8 (62.5%)
Tubulitis	0/9 (0%)	5/8 (62.5%)
Interstitial		
Monocytes infiltration	3/9 (33%)	7/8 (87.5%)
Fibrosis	0/9 (0%)	6/8 (75%)
Cortical scarring (fibrosis)	0/9 (0%)	7/8 (87.5%)

litis and interstitial monocyte infiltration, were observed in 20% of the isografted kidneys.

Immunohistochemically, both ET-1 and ET-3 were present in the isografts at relatively low levels. ET-1 was found in the proximal and in low concentration in the distal convoluted tubules, endothelium and smooth muscle cells of the large blood vessels (Fig. 1). The distribution of ET-3 immunoreactivity was similar to that of ET-1, except that in the glomeruli ET-3 was stronger in the epithelial cells whereas ET-1 protein expression was stronger in the endothelial cells (Fig. 1). The mesangial cells showed low immunoreactivity for both peptides. In addition, the collecting ducts showed positive immunoreactivity for both peptides. Increased immunoexpression of ET-1 and ET-3 were observed in the allografted kidneys, but the distribution of immunoreactivity was similar compared to that of isografts. Similarly, increased immunoreactivity of ET-3 was found in the allografts, mostly in the vascular smooth muscle cells and tubular cells. It is of interest to note that sclerotic vessels in the allografts showed further increased ET-1 and ET-3 expression in the areas of sclerosis (Fig. 1).

Sandwich enzyme immunoassays for ET-1 and ET-3 revealed that ET-1 was the predominant peptide in rat grafted kidneys. The concentrations of ET-1 were 109 ± 40 and 331 ± 54 pg/g wet tissue for isografts and allografts, respectively showing a three-fold increase in ET-1 expression was noted ($P < 0.05$, Student t-test). The concentrations of ET-3 in rat grafted kidneys were at the low range (0–40 pg/g wet tissue) of the ELISA standard. They were 20.71 ± 1.52 and 34.35 ± 3.50 pg/g wet tissue for isografts and allografts, respectively. The ET-3 expressions in allografts were significantly elevated as compared to that in isografts ($P < 0.05$).

In order to define the optimal linear amplification conditions for each primer set, a series of pilot studies were performed using various amounts of RT products and different cycle numbers of PCR amplification. The levels of the mRNA expression of ET-1, ET-3 were significantly raised in allografts compared to isografts ($P < 0.05$), as shown in Fig. 3. On the other hand, a significant downregulation of ET_A mRNA was noted in the allografts compared to isografts. The ET_B mRNA expression remained unchanged between the allografts and isografts (Figs. 2, 3).

Discussion

The results of solid organ transplantation have been consistently improving as a result of better tissue typing, refined surgical techniques, and good immunosuppresive drugs. However, a significant portion of grafts will eventually fail due to chronic rejection [27]. Chronic renal allograft rejection has been defined as a progressive functional deterioration occurring months or years after engraftment. Both immunological and nonimmunological factors have been associated with chronic rejection [8, 10, 27]. Despite different factors that may contribute to the rejection, the pathological lesions remain consistent, including glomerulopathy and vascular abnormalities. Here we have reproduced an animal model in which histological lesions of chronic rejection as demonstrated in the rat model were observed [8]. The allografted animals showed significantly high serum creatinine levels and proteinuria that indicating impaired renal function. It is however to be noted that the serum creatinine levels in isografts were relatively high, suggesting some degree of renal injury in the isografts as previously described in this model [8].

In this study, we have shown that ET-1 is the predominant peptide isoform in the kidney. However both protein and mRNA of ET-1 and ET-3 were detected and found to be elevated in the allografts. Immunohistochemically ET-1 was present in isografted tubular epithelial cells, vasculature and glomerular epithelial cells. This distribution is similar to what has been reported previously by Wilkes et al. [34]. Similarly, immunoreactive ET-3 was found in collecting ducts, glomeruli, and tubules and in the vasculature. Within the glomeruli, the immunoreactive ET-1 was predominantly localized in endothelial cells, whereas the ET-3 immunoreactivity was more prominent in epithelial cells. This heterogeneous distribution of ET-1 and ET-3 in kidneys suggests that the two peptides may be involved in different renal functions.

We have demonstrated significantly increased ET-1 and ET-3 immunoreactivity and mRNA expression in allografted kidneys compared to the isografts. Previous studies have shown that in acute vascular rejection, there was a significant increase in the serum ET-1 level with reduced ET-1 immunoreactivity of the endothelium of renal vasculature [33]. On the other hand in acute

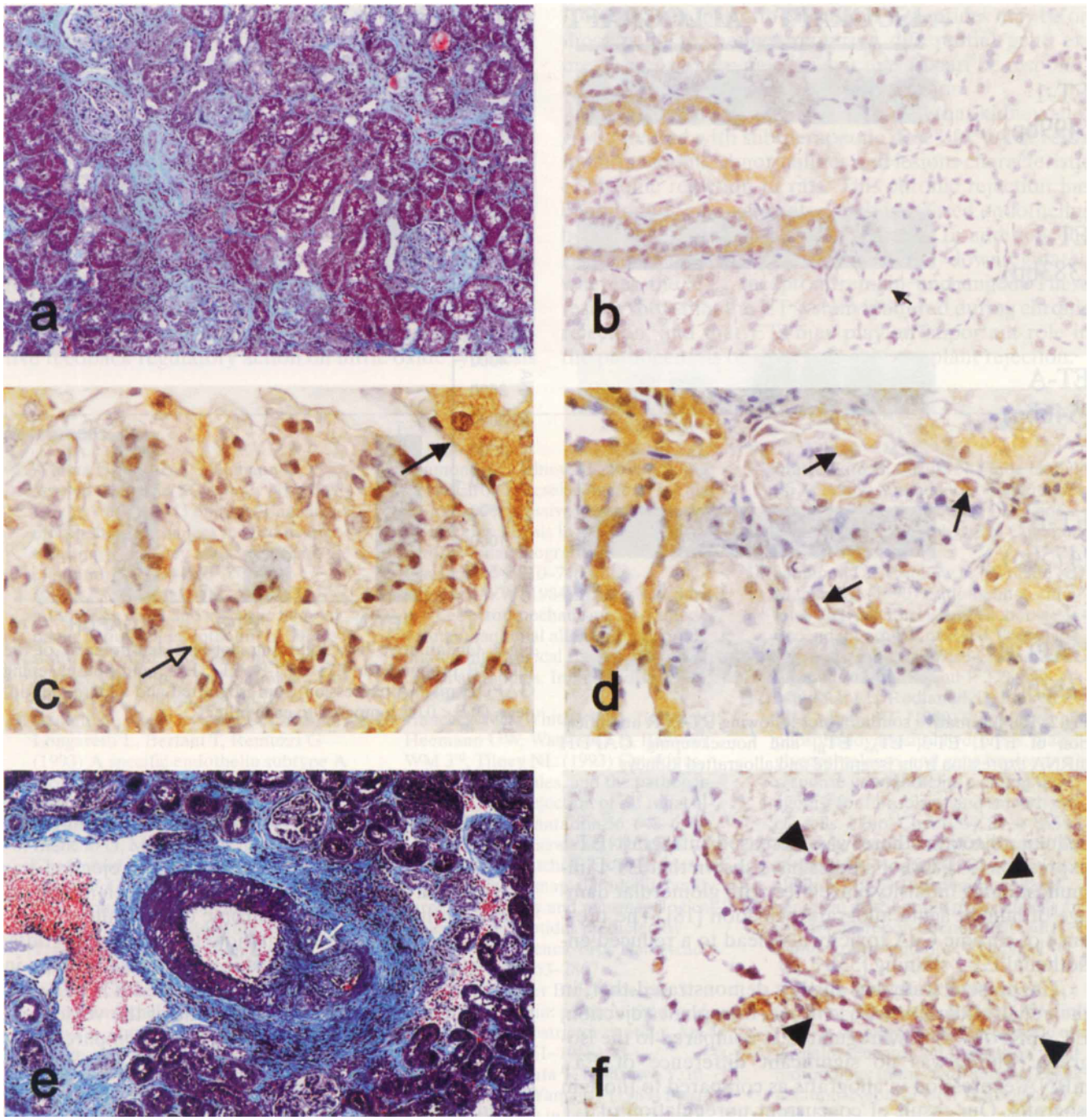

Fig. 1 Sections of rat kidneys with **a** Trichrome stain showing abundant collagen deposits in the glomeruli and interstitial fibrosis in an allograft. **b** ET-1 immunoreactivity in the tubule and in the glomerular endothelium (*arrow*) in an isograft **c** ET-1 immunoreactivity in an allograft showing strong positive immunoreactivity in the endothelial cells (*open arrow*) and in the tubules (*arrow*) **d** ET-3 immunostain, showing intense immunoreactivity in the epithelial cells of the glomerulus of an allograft (*arrow*). **e** An artery from an allograft with elastic-trichrome stain showing segmental intimal proliferation. *Open arrow* Elastic fiber indicating the internal limiting membrane). **f** High power view of an artery from the allograft showing intense immunostaining of ET-1 in the neointimal lesion (*arrow head*) compared to the weak immunoreactivity in the relatively normal area (*left side*)

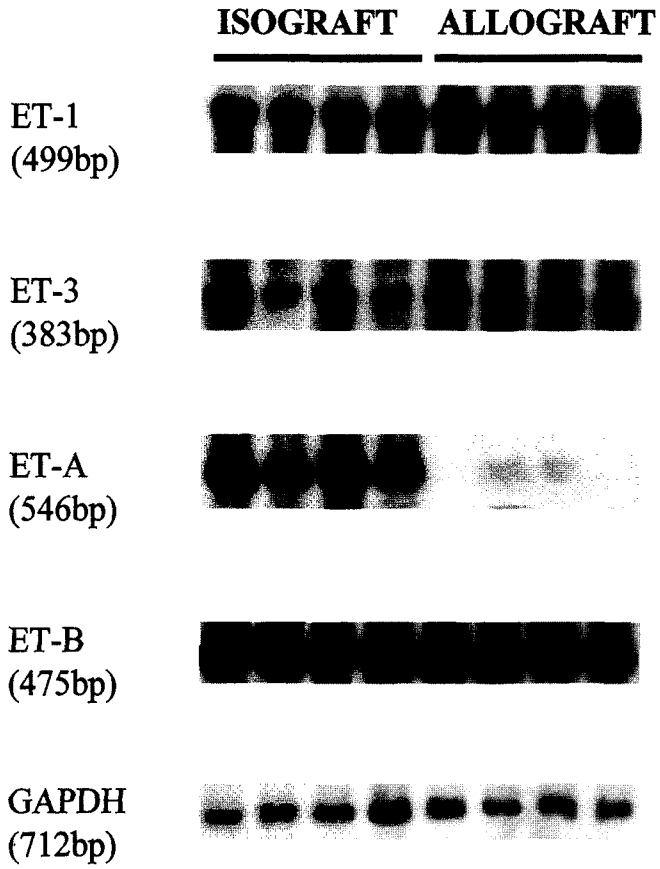

Fig. 2 Representative southern blot following RT-PCR amplification of ET-1, ET-3, ET_A, ET_B, and housekeeping GAPDH mRNA expression from isografted and allografted kidneys

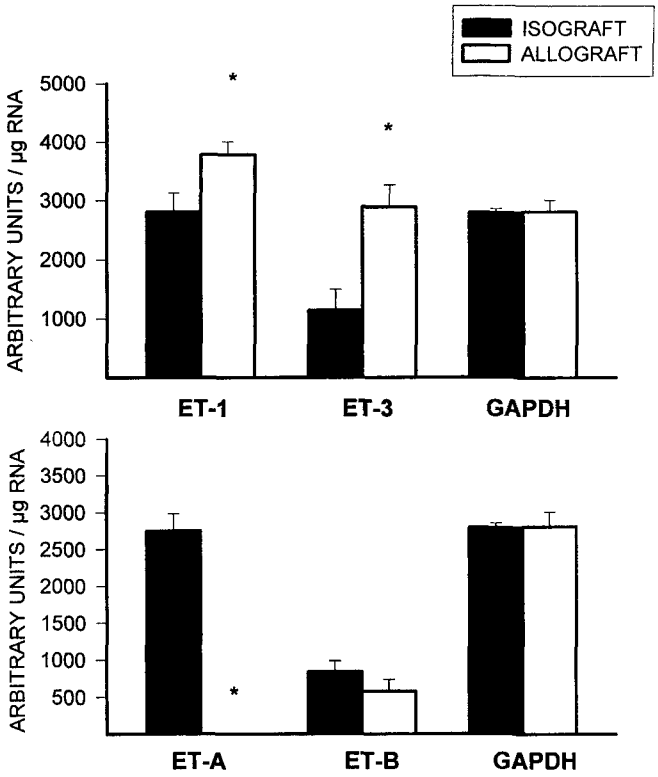

Fig. 3 Quantitative data following slot-blot densitometry of the RT-PCR products for ET-1, ET-3, ET_A, ET_B, and housekeeping GAPDH mRNA expression from isografted and allografted kidneys. (* $P < 0.05$ compared to each other)

cellular rejection, there was increased intragraft ET-1 expression [33]. Other studies have shown that ET-1 immunostaining intensity correlated with glomerular damage of human acute allografts rejection [18]. The presence of chronic CyA toxicity also lead to a reduced endothelial ET-1 staining [33].

The present study has further demonstrated that, in the renal allografts with chronic transplant rejection, ET_A mRNA was downregulated as compared to the isografts. There was no significant difference of ET_B mRNA expression in allografts as compared to those in isografts. Our result of concurrent upregulation of ET peptides and downregulation of ET receptors is in agreement with the notion that ET is regulated through an autocrine/paracrine fashion. Although our results may suggest that substantial ET related effects in this model have been mediated through ET_B receptor, ET_A receptor blockade has been sucessful in attenuating transplant glomerulopathy [21]. Alteration of ET receptor expression has been reported in several pathophysiological conditions and possibly contributes to disease processes. [19, 23]. These results suggest that components of ET system may play different roles in various pathological conditions, leading to the development of renal failure.

The important role of ETs in chronic rejection has further been shown in a recent study in which inhibition of ET synthesis prevented chronic cardiac allograft rejection [26]. It is of interest to note that in the allografts, the hyperplastic neointima exhibited intense staining in the smooth muscle cells for both ET-1 and ET-3. In contrast, the media of uninjured sections of the vessels exhibited reduced immunoreactivity when compared with the neointima. This finding suggests that upregulation of ET expression is associated with the development of vascular obliteration seen in the chronic allograft rejection. In a recent study on human chronical renal allograft rejection, increased neointimal ET-1 has been demonstrated [25].

However, factors that mediate the increased expression of ET-1 and ET-3 in the rejection lesions are unknown. Possibly ET peptides are induced by a variety of cytokines which are released as part of the immune response of the recipient to the allograft. Infiltrating activated mononuclear cells secrete a variety of cytokines [4, 9], which may act synergistically to cause increased

ET-expression depending on their temporal interplay [31]. However, further studies are needed in determining which factor, or factors, are specific in triggering the ET secretions by donor tissue in transplant vasculopathy and chronic rejection.

The exact role(s) of ET peptides in chronic rejection processes remain unclear, although previous studies in animal models and in humans with chronic allograft rejection implicate the mitogenic and vasopressor actions of ET peptides in transplant vasculopathy. The elevated ET-1 and ET-3 may cause vasoconstriction and decrease renal plasma flow and glomerular filtration rate [12, 24]. ET has been shown to interact with other potent vasoactive substances such as nitric oxide and possesses a positive feedback regulatory action on nitric oxide synthesis [24, 28]. The long-term effects of ET-peptides may be on those cellular changes requiring differential gene expression. All these mechanisms may in part be responsible for the production of chronic rejection.

In summary, we have demonstrated that kidney allografts treated with subtherapeutic dose of CyA develop renal functional abnormalities and lesions characteristic of chronic rejection in rats. This chronic rejection has been found to be associated with increased endothelins both at the protein and mRNA levels. In addition, the ET_A receptor has been found to be downregulated whereas the ET_B receptors remain unchanged. These results show that the ET system is altered during chronic rejection, and that ETs may play an important role in the pathogenesis of chronic renal transplant rejection.

References

1. Azuma H, Binder J, Heemann U, Schmid C, Tullius SG, Tilney NL (1995) Effects of RS61443 on functional and morphological changes in chronically rejecting rat kidney allografts. Transplantation 59: 460–466
2. Banks RO, Pollock DM, Novak J (1997) The renal and systemic hemodynamic actions of endothelin. In: Highsmith RF (ed) Endothelin: molecular biology, physiology, and pathology. Humana Press, Totowa, NJ, pp 167
3. Benigni A, Zoja C, Corna D, Orisio S, Longaretti L, Bertani T, Remuzzi G (1993) A specific endothelin subtype A receptor antagonist protects against injury in renal disease progression. Kidney Int 44: 440–444
4. Blancho G, Moreau JF, Chabannes D, Chatenoud L, Soulillou JP (1993) HIL-DA/LIF, G.CSF, IL-1 beta, IL-6, and TNF alpha production during acute rejection of human kidney allografts. Transplantation 56: 597–602
5. Chakrabarti S, Gan XT, Merry A, Karmazyn M, Sima AA (1998) Augmented retinal endothelin-1, endothelin-3, endothelin$_A$ and endothelin$_B$ gene expression in chronic diabetes. Curr Eye Res 17: 301–307
6. Chakrabarti S, Sima AA (1997) Endothelin-1 and endothelin-3-like immunoreactivity in the eyes of diabetic and non-diabetic BB/W rats. Diabetes Res Clin Pract 37: 109–120
7. Deray G, Carayon A, Maistre G, Benhmida M, Masson F, Barthelemy C, Petitclerc T, Jacobs C (1992) Endothelin in chronic renal failure. Nephrol Dial Transplant 7: 300–305
8. Diamond JR, Tilney NL, Frye J, Ding G, McElroy J, Pesek-Diamond I, Yang H (1992) Progressive albuminuria and glomerulosclerosis in a rat model of chronic renal allograft rejection. Transplantation 54: 710–716
9. Hancock WW (1984) Analysis of intragraft effector mechanisms associated with human renal allograft rejection: immunohistological studies with monoclonal antibodies. Immunol Rev 77: 61–84
10. Hancock WH, Whitley WD, Tullius SG, Heemann UW, Wasowska B, Baldwin WM 3rd, Tilney NL (1993) Cytokines, adhesion molecules, and the pathogenesis of chronic rejection of rat renal allografts. Transplantation 56: 643–650
11. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T (1989) The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci U S A 86: 2863–2867
12. King AJ, Brenner BM, Anderson S (1989) Endothelin: a potent renal and systemic vasoconstrictor peptide. Am J Physiol 256:F1051–1058
13. Koyama H, Tabata T, Nishzawa Y, Inoue T, Morii H, Yamaji T (1989) Plasma endothelin levels in patients with uraemia. Lancet 1: 991–992
14. Kurihara Y, Kurihara H, Suzuki H, Kodama T, Maemura K, Nagai R, Oda H, Kuwaki T, Cao WH, Kamada N (1994) Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. Nature 368: 703–710
15. Matsumoto H, Suzuki N, Onda H, Fujino M (1989) Abundance of endothelin-3 in rat intestine, pituitary gland and brain. Biochem Biophys Res Commun 164: 74–80
16. Muldoon LL, Pribnow D, Rodland KD, Magun BE (1990) Endothelin-1 stimulates DNA synthesis and anchorage-independent growth of Rat-1 fibroblasts through a protein kinase C-dependent mechanism. Cell Regul 1: 379–390
17. Muldoon LL, Rodland KD, Forsythe ML, Magun BE (1989) Stimulation of phosphatidylinositol hydrolysis, diacylglycerol release, and gene expression in response to endothelin, a potent new agonist for fibroblasts and smooth muscle cells. J Biol Chem 264: 8529–8536
18. Murer L, Zacchello G, Dall'Amico R, Masiero M, Montini G, Basso G, Zacchello F (1997) Renal immunohistochemical distribution, plasma levels and urinary excretion of endothelin after kidney transplantation. J Nephrol 10: 318–324
19. Nabokov A, Amann K, Wagner J, Gehlen F, Munter K, Ritz E (1996) Influence of specific and non-specific endothelin receptor antagonists on renal morphology in rats with surgical renal ablation. Nephrol Dial Transplant 11: 514–520
20. Orisio S, Benigni A, Bruzzi I, Corna D, Perico N, Zoja C, Benatti L, Remuzzi G (1993) Renal endothelin gene expression is increased in remnant kidney and correlates with disease progression. Kidney Int 43: 354–358

21. Orth SR, Odoni G, Amann K, Strzelczyk P, Raschack M, Ritz E (1999)The ET(A) receptor blocker LU 135252 prevents chronic transplant nephropathy in the "Fisher to Lewis" model. J Am Soc Nephrol 10: 387–391
22. Peri A, Vannelli GB, Fantoni G, Giannini S, Barni T, Orlando C, Serio M, Maggi M (1992) Endothelin in rabbit uterus during pregnancy. Am J Physiol 263:E158–167
23. Pollock DM, Polakowski JS (1997) ETA receptor blockade prevents hypertension associated with exogenous endothelin-1 but not renal mass reduction in the rat. J Am Soc Nephrol 8: 1054–1060
24. Simonson MS (1993) Endothelins: multifunctional renal peptides. Physiol Rev 73: 375–411
25. Simonson MS, Emancipator SN, Knauss T, Hricik DE (1998) Elevated neointimal endothelin-1 in trasplantation-associated arteriosclerosis of renal allograft recipients. Kidney Int 54: 960–971
26. Simonson MS, Herman WH, Robinson A, Schulak J, Hricik DE (1999) Inhibition of endothelin-converting enzyme attenuates transplant vasculopathy and rejection in rat cardiac allografts. Transplantation 67: 1542–1547
27. Tilney NL, Whitley WD, Diamond JR, Kupiec-Weglinski JW, Adams DH (1991) Chronic rejection–an undefined conundrum. Transplantation 52: 389–398
28. Vanhoutte PM (1994) Endothelin-1. A matter of life and breath. Nature 368: 693–694
29. Warrens AN, Cassidy MJ, Takahashi K, Ghatei MA, Bloom SR (1990) Endothelin in renal failure. Nephrol Dial Transplant 5: 418–422
30. Watschinger B, Sayegh MH, Hancock WW, Russell ME (1995) Up-regulation of endothelin-1 mRNA and peptide expression in rat cardiac allografts with rejection and arteriosclerosis. Am J Pathol 146: 1065–1072
31. Watschinger B, Sayegh MH (1996) Endothelin in organ transplantation. Am J Kidney Dis 27: 151–161
32. Watschinger B, Vychytil A, Schuller M, Hartter E, Traindl O, Pohanka E, Ulrich E, Kovarik J (1991) The pathophysiologic role of endothelin in acute vascular rejection after renal transplantation. Transplantation 52: 743–746
33. Watschinger B, Vychytil A, Attar M, Wagner D, Schuller M, Hartter E, Ulrich W (1994) Pattern of endothelin immunostaining during rejection episodes after kidney transplantation. Clin Nephrol 41: 86–93
34. Wilkes BM, Susin M, Mento PF, Macica CM, Girardi EP, Boss E, Nord EP (1991) Localization of endothelin-like immunoreactivity in rat kidneys. Am J Physiol 260:F913–920