Comprehensive immunohistological analysis of the endothelin system in human kidney grafts

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Abstract
Background. In experimental models of renal transplantation, upregulation of the endothelin (ET) system and amelioration of renal injury by ET-receptor blockers have been documented. In contrast, little information is available on the expression of the ET system in human kidney allografts. It was the purpose of the present study to analyse by immunohistology the expression of ET-1 as well as of the two ET receptors (ET-RA and ET-RB) in the different cells and compartments of kidney grafts and control kidneys.

Methods. Fifty-five graft biopsies were taken from 55 kidney allograft recipients (mean age: 32±2.8 years) who were all on a calcineurin inhibitor. The indication for biopsies was delayed graft function or suspected rejection. The underlying diagnoses were acute allograft rejection (n=14), chronic allograft nephropathy (n=14), cyclosporin A (CSA) toxicity (n=10), post-operative acute tubular necrosis (ATN) (n=11) and recurrent primary disease (n=6). As control, tissues of non-grafted kidneys with ATN (mean age: 35±24 years), of primary glomerulonephritis (mean age: 69±10 years) and of non-tumour-bearing parts of eight tumour nephrectomy specimens (mean age: 67±5 years) were assessed. The biopsies were scored using the 1997 Banff criteria. Expression of ET-1, ET-RA and ET-RB as well as of vascular endothelial growth factor was evaluated by immunohistochemistry and a semi-quantitative scoring system. Interstitial infiltrating cells were characterized using antibodies against T cells, B cells and macrophages (CD3, CD20 and CD68).

Results. Control cases showed only faint expression of ET-1 in glomeruli (in podocytes and endothelial cells), whereas marked expression was seen in distal, but less in proximal tubular cells. The interstitium was completely negative. ET-1 expression was seen in vascular endothelial cells (VEC) and vascular smooth muscle cells (VSMC). Only faint expression of ET-RA and ET-RB was found in glomeruli and tubuli (distal more than proximal). Marked ET-RA and ET-RB expression was seen in VEC and VSMC. In all transplanted kidneys, irrespective of the underlying diagnosis, expression of ET-1, ET-RA and ET-RB was markedly higher compared with control kidneys. ET-1 was strikingly upregulated in glomeruli and tubuli, but surprisingly not in the vasculature of grafts with CSA toxicity. Expression of ET-RB was markedly increased in CSA toxicity in glomeruli, tubuli and vessels. In grafts with ATN and acute rejection, pronounced expression of ET-RA was noted. There was a strong correlation between proteinuria and expression of ET-1 in glomeruli and proximal tubuli and of ET-RB in proximal tubuli.

Conclusions. The above data in human kidney allograft biopsies are consistent with an important role of the ET system in different types of renal allograft damage. This finding extends and clarifies the somewhat contradictory results in animal models.

Keywords: acute renal failure; endothelin; endothelin receptor; recurrent glomerulonephritis; transplantation

Introduction

Studies in experimental models of renal [1] and non-renal [2] allografts documented increased expression of endothelin (ET) as well as attenuation of renal damage with the use of selective ETA-receptor blockers [3,4], but not after administration of non-selective...
ET-receptor blockers [5]. These observations raised considerable interest in the regulation of the ET system in human allografts. They could also provide a potential rationale for therapeutic use of ET-receptor antagonists. Unfortunately, the experimental and human information available has thus for remained incomplete and controversial.

Murer et al. [6] examined ET-1 plasma concentrations and ET-1 excretion in the urine of patients with renal allografts. In addition, they also examined the expression of ET-1 in glomeruli, infiltrating cells, vessels and tubuli of the allografts. The authors noted a correlation between ET-1 expression and glomerular lesions (i.e. proliferation and periglomerular fibrosis). No correlation was noted however, between vascular lesions and the intensity of ET-1 expression in the vessel wall. Simonson et al. [7] analysed vascular expression of ET-1 in renal transplant patients with chronic (n = 12) or acute rejection (n = 11) compared with normal controls. They found an upregulation of vascular ET-1 in renal allografts with chronic rejection. Chareandee et al. [8] assessed tubular ET-1 expression in 18 renal allograft recipients undergoing acute rejection, seven patients with chronic allograft nephropathy (CAN) and five normal kidneys. In patients with allograft rejection, ET-1 expression in proximal and distal tubuli was increased. In parallel, in vitro an upregulation of ET-1 secretion was observed in primary cultures of proximal tubular epithelium cells [8].

In contrast, Watschinger et al. [9] found less intense staining for ET in biopsies of allografts undergoing acute vascular rejection, but staining was similar to control kidneys in grafts undergoing interstitial rejection and acute tubular necrosis (ATN). Chronic cyclosporin A (CSA) toxicity was associated with reduced staining for ET. The authors concluded that endothelial cell damage reduced ET staining [9].

Such controversial findings in animal and human studies, i.e. differences with respect to localization or intensity of staining as well as different associations with graft pathology, are presumably the result of differences in methodology and species.

The tantalizingly positive effects of ET-receptor blockade in experimental transplantation [3,4] motivated us to perform a comprehensive analysis of expression of the different components of the ET system in human allografts with different pathologies, i.e. ATN, CSA toxicity, acute rejection and CAN.

**Subjects and methods**

**Patient data**

The 55 graft biopsies were taken from 55 kidney allograft recipients [24 female, 31 male; mean age: 32 ± 2.8 years (range: 2–67 years)] (Table 1). Eight tumour nephrectomy specimens served as controls. Samples were taken from the non-tumour-bearing parts of kidneys with renal cell carcinoma [four female, four male; mean age: 67 ± 5 years (range: 62–76 years)]. All kidney allograft recipients were on a calcineurin inhibitor [CSA (n = 5) or tacrolimus (n = 4)]. Forty-five patients had triple immunosuppressive therapy with azathioprine and steroids in addition to the calcineurin inhibitor. Six patients took a calcineurin inhibitor and steroids, three were on a calcineurin inhibitor and azathioprine and one patient took only a calcineurin inhibitor.

Blood pressure data were available from 51 patients. Nine patients had normal blood pressure without any antihypertensive medication, whereas 42 patients were hypertensive (> 140/90 mmHg). Eleven of these patients were on a monotherapy [seven with β-blockers, two with calcium-channel blockers, one with an angiotensin-converting enzyme (ACE) inhibitor and one with a diuretic]; 21 patients received a combination of two antihypertensive agents, most of them a β-blocker and a diuretic, but also β-blockers and calcium-channel blockers or β-blockers and angiotensin II-receptor blockers. Seven patients were on triple antihypertensive therapy and three patients each received four antihypertensive agents.

The indications for biopsies were delayed graft function or suspected rejection. The diagnoses were acute allograft rejection (n = 14), CAN (n = 14), CSA toxicity (n = 10), post-operative ATN (n = 11) and recurrent primary disease (n = 6).

**Table 1. Relevant patient characteristics in the different diagnostic groups**

<table>
<thead>
<tr>
<th></th>
<th>Acute rejection (n = 14)</th>
<th>Chronic allograft nephropathy (n = 14)</th>
<th>CSA toxicity (n = 10)</th>
<th>Recurrent disease (n = 6)</th>
<th>Post-operative ATN (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (years)</td>
<td>34 ± 6 (7–67)</td>
<td>15 ± 2 (2–23)</td>
<td>28 ± 5 (12–55)</td>
<td>48 ± 9 (20–66)</td>
<td>43 ± 6 (3–65)</td>
</tr>
<tr>
<td>Duration of dialysis (years)</td>
<td>3.3 ± 0.9</td>
<td>1.8 ± 0.5</td>
<td>3.5 ± 1</td>
<td>2.8 ± 1.3</td>
<td>4.8 ± 0.9</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>46 ± 5</td>
<td>35 ± 6</td>
<td>40 ± 7</td>
<td>36 ± 10</td>
<td>58 ± 3</td>
</tr>
<tr>
<td>Time since transplantation (months)</td>
<td>4 ± 2</td>
<td>51 ± 9</td>
<td>32 ± 12</td>
<td>78 ± 37</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td>Cold ischaemia time (h)</td>
<td>12 ± 3</td>
<td>16 ± 3</td>
<td>16 ± 3</td>
<td>17 ± 5</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>2.8 ± 0.5</td>
<td>2.9 ± 0.2</td>
<td>2.8 ± 0.4</td>
<td>1.7 ± 0.3</td>
<td>5.1 ± 0.8</td>
</tr>
<tr>
<td>Serum urea (mg/dl)</td>
<td>102 ± 10</td>
<td>79 ± 8</td>
<td>108 ± 20</td>
<td>65 ± 12</td>
<td>167 ± 23</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.3</td>
<td>3 ± 3</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>Mean systolic and diastolic blood pressure (mmHg)</td>
<td>138/91 ± 11/6</td>
<td>136/89 ± 11/6</td>
<td>143/94 ± 5/2</td>
<td>138/90 ± 18/9</td>
<td>141/94 ± 3/2</td>
</tr>
<tr>
<td>Number of antihypertensive medications</td>
<td>1.5 ± 1.1 (0–3)</td>
<td>1.1 ± 1.0 (0–3)</td>
<td>2.1 ± 0.7 (1–3)</td>
<td>1.8 ± 1.8 (0–4)</td>
<td>2 ± 0.5 (1–3)</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD (range).
Biopsies of non-grafted kidneys with ATN \(n=4\); two male, two female; mean age: 35 ± 24 years (range: 10–67 years) and primary glomerulonephritis \(GN; \ n=4\); one male, three females; mean age: 69 ± 10 years (range: 60–80 years) were examined as well as controls.

Evidence of acute or chronic allograft rejection, post-operative ATN, CSA toxicity or recurrent disease was scored using the 1997 Banff criteria [10].

In order to analyse the effect of cold ischaemia time on expression of the ET system in the kidney, patients were divided into two groups according to cold ischaemia time <0.5 and >0.5 g/day. In order to analyse the effect of proteinuria on expression of the ET system in the kidney, patients were divided into two groups according to proteinuria <0.5 and >0.5 g/day.

**Immunohistological investigations**

Endothelin 1 (ET-1) and ET receptors A (ET-RA) and B (ET-RB) protein expression was analysed by immunohistochemistry (biotin–streptavidin amplification system) of paraffin sections using a mouse anti-human ET-1 antibody (polyclonal, MA3-005; ABR Co., CO, USA), a sheep anti-human ET-RA antibody (polyclonal; Biotrend Co., Cologne, Germany) and a sheep anti-human ET-RB antibody (polyclonal; Research Diagnostic Inc., NJ, USA). In order to provide a histological index of the severity of proteinuria the samples were stained with an antibody against albumin (rabbit anti-human, polyclonal; Abcam Co., UK). Due to the well-known interaction of ET-1 and vascular endothelial growth factor (VEGF) protein, expression of VEGF was also analysed using a rabbit anti-human VEGF antibody (polyclonal; Genzyme Co., Cambridge, MA, USA). In addition, in order to characterize the infiltrating mononuclear inflammatory cells in greater detail, the following antibodies were used: CD3 [mouse anti-human CD3 antibody, T-cell (clone F7.2.38), monoclonal], CD20 [mouse anti-human CD20 antibody, B-cell (clone L26), monoclonal], CD20 [mouse anti-human CD68 antibody (clone PG-M1), macrophage, monoclonal] (DAKO, Glostrup, Denmark).

The kidney samples were fixed in 10% buffered formalin, embedded in paraffin, cut into 4 μm sections, heat-fixed (65°C, 30 min) and deparaffinized. Deparaffinized sections were washed in phosphate-buffered saline (PBS). Antigen retrieval solutions, blocking solutions, dilution of antibodies and incubation times are given in Table 2. Optimal concentrations of primary antibodies were established in dilution series. As a secondary antibody, super-sensitive multi-link (anti-lg for mouse, rabbit, guinea pig, rat-AB; BioGenex, San Ramon, CA, USA) (ET-1, VEGF, CD3, CD20, CD68) or biotin–streptavidin-conjugated pure rabbit anti-sheep IgG (Jackson Immunoresearch, West Baltimore, USA), 1:5000 (ET-RA and ET-RB) were used for 30 min at room temperature. Alkaline phosphatase-conjugated streptavidin in PBS (BioGenex, San Ramon, CA, USA) was used for labelling. Binding of the primary antibodies was visualized using the Fast Red Substrate System (DAKO, Glostrup, Denmark), which consists of a specific tablet (fast red, naphthol phosphate and levamisole) and substrate buffer. Sections were counterstained with Mayer’s hemalaun. Negative controls (omission of the primary antibody) were consistently carried out.
**Semi-quantitative grading.** Immunohistochemical staining for ET-1, ET-RA, ET-RB and albumin was graded by an investigator who was unaware of the underlying diagnosis using the following scale: 0 = no staining, 1 = mild staining, 2 = moderate staining, 3 = intense staining. Expression of VEGF was graded 0 = no staining, 1 = mild staining, 2 = moderate staining, 3 = intense staining, 4 = extremely intense staining. Immunostaining for these epitopes was analysed in different tissue compartments, i.e. glomeruli, tubuli, interstitium and vasculature. Expression of CD3, CD20 and CD68 was evaluated by counting the number of positive cells per field of view at a magnification of ×200.

**Statistics**

Data are expressed as means±SD (range) or median and range, as indicated. Data were analysed by using the non-parametric Mann–Whitney test or Kruskal Wallis test, as indicated. The results were considered significant when the probability of error (P) was <0.05.

**Results**

**Expression of the components of the ET system**

**Control kidneys.** Faint expression of ET-1 (Figure 1A) was seen in glomeruli whereas expression was higher in distal but not proximal tubular cells. The interstitium was completely negative. Marked ET-1 expression was also seen in vascular endothelial cells (VEC) and vascular smooth muscle cells (VSMC). Expression of ET-RA (Figure 1E) and ET-RB (Figure 1I) was faint in glomeruli and tubuli (distal more than proximal). More pronounced ET-RA and ET-RB expression was seen in VEC and VSMC.

**Acute rejection.** Expression of ET-1 was increased in glomeruli, but more so in tubuli and in VEC and VSMC (Figure 1B). In parallel, glomerular and tubular ET-RA expression was also higher than in controls (Figure 1F). The expression of ET-RB was less markedly increased, but still more intense than in controls (Figure 1J).

**Chronic allograft nephropathy.** Expression of ET-1 (Figure 1C), ET-RA (Figure 1G) and ET-RB (Figure 1K) in glomeruli and tubuli was more intense than in controls. Marked expression of ET-RA and ET-RB was also seen in vessels. Expression of ET-RA in CAN was less intense than in acute rejection and post-operative ATN, but still more marked than in controls.

**CSA toxicity.** Marked ET-1 expression was seen in the glomeruli, predominantly in podocytes and some mesangial cells. ET-1 expression was also increased in proximal and distal tubuli compared with controls (Figure 1D). The low level expression of ET-1 in the vessels did not significantly differ from that seen in controls. Glomerular and tubular expression of ET-RA and ET-RB was also increased (Figures 1H and 1L).

**Grafts with post-operative ATN.** Expression of ET-1 was pronounced in glomeruli (predominantly in podocytes and endothelial cells), but only faint in tubuli (Figure 1M). Expression of ET-RA (Figure 1N) and ET-RB (Figure 1O) was intense in glomeruli and tubuli. ET-RA expression was also pronounced in the renal vasculature. In non-grafted kidneys with ATN the expression of ET-1 and ET-RA was only faint, but the expression of ET-RB was intense in glomeruli, tubuli and vessels.

**Recurrent glomerulonephritis.** In recurrent GN, glomerular (i.e. podocytes) and tubular expression of ET-1 (Figure 1P), of ET-RA and particularly of ET-RB was markedly higher than in controls (while it was much less marked in primary GN). In contrast, expression was not increased in the interstitium and in the vessels. The semiquantitative analyzes or glomerular and tubular expression of the components of the ET-system are given in Table 3 and Figure 3.

**Expression of VEGF in different compartments of the kidney**

Glomerular expression of VEGF was very low in controls (Figure 1Q). The expression was significantly more marked in acute (Figure 1R) and chronic rejection (Figure 1S) as well as in CSA toxicity (Figure 1T). In chronic rejection, glomerular expression was significantly more intense than in acute rejection. In the tubulointerstitium hardly any VEGF expression was noted in control kidneys, but a significant upregulation was found in acute and chronic rejection (Figure 4).

A negative correlation was found between glomerular VEGF expression and cold ischaemia time. The mean score of glomerular VEGF expression was 2.1 in patients with cold ischaemia time <11 h (n = 11) and 1.4 in those with cold ischaemia time >11 h (n = 26) (P < 0.05).

**Correlation of expression of components of the ET system with proteinuria and cold ischaemia time**

**Proteinuria.** A significant correlation was noted between proteinuria and expression of ET-1 (in glomeruli and proximal tubuli) as well as of ET-RB (in proximal tubuli) when the findings in all 55 patients were combined (Figure 3).

**Ischemia time.** Overall, a high expression of glomerular ET-1 was noted in grafts with cold ischaemia time >11 h. The mean score of glomerular ET-1 expression was 0.2 in patients with cold ischaemia time <11 h (n = 16) and 0.8 in those with cold ischaemia time >11 h (n = 28) (P < 0.005).

**Patient age and gender.** No correlation was found between the age or gender of patients and ET-1 or ET-receptor expression, respectively. A correlation was found, however, between donor age and ET-1, but not ET-receptor expression. If all 55 patients were combined, higher glomerular, tubular and vascular ET-1 expression (P < 0.05) was found in the grafts obtained...
from donors between 0 and 30 years \((n = 11)\) compared with donors between 31 and 74 years \((n = 44)\). No correlation was found between donor age or gender and ET-1 or ET-receptor expression, respectively.

Characterization of mononuclear interstitial cell infiltrates

\(T\) and \(B\) cells. Practically no \(CD3\)- or \(CD20\)-positive \(T\) or \(B\) cells were seen in the glomeruli of control kidneys and of kidney allografts (Table 4). In the tubulointerstitium of controls, only sporadic \(CD3\)- or \(CD20\)-positive \(T\) or \(B\) cells were seen. Their number was significantly higher in acute and chronic rejection. In addition, the number of \(CD3\)-positive cells was higher in acute rejection than in chronic rejection; the same was true for \(CD20\)-positive cells. A higher number of \(CD3\)- and \(CD20\)-positive cells was seen in CAN IIIb than in CAN I or II. Compared with controls, the number of \(T\) and \(B\) cells was not significantly higher in CSA toxicity, post-operative ATN and recurrent disease.
No CD68-positive macrophages were found in the glomeruli and tubulointerstitium of control kidneys. Only isolated CD68-positive cells were seen in glomeruli of allografts. In contrast, in acute and chronic rejection as well as in post-operative ATN and recurrent disease the number of positive cells in the tubulointerstitium was significantly higher. The highest number of CD68-positive cells was found in acute rejection. Many CD68-positive macrophages were also seen in CAN, post-operative ATN and recurrent disease. No correlation was seen, however, between the number of CD68-positive cells and the Banff grade of chronic rejection.

Table 3. Semi-quantitative analysis of glomerular and tubular expression of ET-1, ET-RA and ET-RB in the different patient groups (scores)

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=8)</th>
<th>Acute rejection (n=14)</th>
<th>Chronic allograft nephropathy (n=14)</th>
<th>CSA toxicity (n=10)</th>
<th>Post-operative ATN (n=11)</th>
<th>Recurrent disease (n=6)</th>
<th>Non-grafted kidneys with ATN (n=4)</th>
<th>Primary GN (n=4)</th>
<th>Kruskal Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1 Glomeruli</td>
<td>0.02±0.1</td>
<td>0.7±0.6a</td>
<td>0.6±0.2a</td>
<td>0.9±0.4a</td>
<td>0.6±0.5a</td>
<td>1.5±0.9a</td>
<td>0.5±0.7a</td>
<td>0.3±0.1a</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>ET-1 Tubuli</td>
<td>0.5±0.2</td>
<td>1.1±0.4a</td>
<td>1.1±0.4a</td>
<td>0.9±0.5a</td>
<td>0.8±0.5</td>
<td>1±0.4</td>
<td>0.7±0.8</td>
<td>0.7±0.5</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>ET-RA Glomeruli</td>
<td>0</td>
<td>1.7±0.6a</td>
<td>1.3±0.5a</td>
<td>1.2±0.5a</td>
<td>1.8±0.4a</td>
<td>1.3±0.5a</td>
<td>0.1±0.1</td>
<td>0.3±0.5</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>ET-RA Tubuli</td>
<td>0</td>
<td>1±0.4a</td>
<td>0.9±0.2a</td>
<td>0.9±0.4a</td>
<td>1.3±0.7a</td>
<td>1.1±0.3a</td>
<td>0</td>
<td>0.6±0.8</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>ET-RB Glomeruli</td>
<td>0.1±0.1</td>
<td>0.8±0.5a</td>
<td>1.0±0.4a</td>
<td>1.1±0.5a</td>
<td>1.0±0.5a</td>
<td>2.4±0.4a</td>
<td>1.7±0.2a</td>
<td>0.6±0.5</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>ET-RB Tubuli</td>
<td>0.2±0.3</td>
<td>0.9±0.4a</td>
<td>0.7±0.4a</td>
<td>1.0±0.6a</td>
<td>1.2±0.7a</td>
<td>1.5±0.5a</td>
<td>1.2±0.5a</td>
<td>0.5±0.5</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Data are expressed as means±SD.
*P<0.05 vs controls.
Discussion

This comprehensive immunohistological analysis of the ET system in different kidney allograft pathologies documents upregulation of ET-1 as well as of ET-RA and ET-RB expression. This finding suggests an important role of the ET system in different types of renal allograft dysfunction.

The findings are in general agreement with observations on the importance of the ET system in experimental models of chronic allograft rejection of the kidney [3,11,12], the aorta [13] and the heart [14]. In the model of orthotopic transplantation of Fisher-to-Lewis rat kidneys we found that the preferential ET-A receptor blocker LU 135252 almost completely prevented chronic transplant nephropathy [3]. The almost complete abrogation of glomerulosclerosis, tubulointerstitial and vascular damage in this model by an ET-receptor blocker was blood pressure-independent and similar in magnitude, but not in addition to that provided by an ACE inhibitor [12]. The absence of renal haemodynamic changes after administration of an ET-receptor blocker suggested [11] that the improved expression of cell surface markers for macrophages, T cells and MHC-II was not mediated by haemodynamic effects. In the normotensive model of orthotopic allotransplantation of the infrarenal abdominal aorta we also found amelioration of chronic transplant vasculopathy [13]. Finally, Simonson et al. [14] found in the Lewis-to-Fisher heterotopic cardiac allograft model that the ET-converting enzyme inhibitor phosphoramidone prolonged graft survival and improved vasculopathy. The beneficial effect of ET-receptor blockers in these experimental models is not unanticipated in view of the observation of Deng et al. [1] that in the allografted kidney the expression of ET-1 is increased at the mRNA and the protein level.

In human kidney grafts, Watschinger et al. [9] documented increased expression of ET-1 in the renal allograft during episodes of acute cellular rejection, while ET-1 immunoreactivity of renal endothelial cells was decreased during episodes of acute vascular rejection. He also noted increased ET-1 expression in chronic CSA toxicity. In agreement with these findings, Murer et al. [6] noted a good correlation between the intensity of immunostaining for ET-1 and the degree of glomerular damage in allograft rejection.

For the interpretation of ET-1 expression it is important to consider confounding factors, particularly proteinuria and cold ischaemia time. In patients with glomerular disease we had shown that proteinuria is correlated with ET-1 expression [15]. In the present study, expression of ET-1 and ET-RB was more intense in graft recipients with heavy proteinuria. It was also more intense in recipients of grafts with cold ischaemia time >11h. Another confounder is acute tubular necrosis (ATN). In the present study ATN was associated with markedly increased ET-1 expression in glomeruli, but not in tubuli, and this is in contrast to the findings in CAN.

In addition to previous studies, the present investigation provides further information on the expression of the different components of the ET systems and their topographic distribution in human allografts. We did not have the opportunity to examine acute vascular rejections which have become rare, where Watschinger et al. [9] had found reduced ET-1 immunoreactivity...
in vessels. Our study clearly confirms, however, his finding of increased concomitant expression of ET-1, ET-A and ET-B in acute cellular rejection and, particularly, in CAN.

In endothelial cell cultures, Willarumsme et al. [16] noted that CSA increased ET-1 expression. This observation is consistent with a role of ET-1 in CSA vasculotoxicity. Against this experimental background it is of note that in kidneys with CSA toxicity ET-1 expression was increased in podocytes, mesangial cells and tubular cells, but less so in capillaries.

In conclusion, the present data argue for an important role of the ET system in acute allograft rejection and CAN. The findings are of interest in view of the experimental observation that blockade of the ET system with ET-A-receptor-specific antagonists prevents the development of CAN in different rat models of renal transplantation [3,4]. Whether this will also be the case in human kidney transplantation requires further studies.

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Conflict of interest statement. None declared.

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