Immunohistochemical Study of Endothelin-1 (ET-1) in Human Acute Renal Allograft Rejection*

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Immunoperoxidase staining was carried out using monoclonal antibody against endothelin-1 on renal allograft biopsy specimens from thirty one patients with acute renal transplant rejection: Banff 97 score IA (n=11), score IB (n=10) and score IIA (n=10). As a control 10 biopsy specimens of the kidneys removed because of trauma were used. Endothelin-1 immunostaining was investigated in the renal interstitial vasculature and tubular epithelium. In the normal kidney the immunoreactivity of endothelin-1 was demonstrated in vascular endothelial cells and tubular epithelial cells. The intensity of endothelin-1 staining in endothelial cells in renal allograft biopsies from patients with Banff score IA and IB was similar to controls. In biopsies with Banff score IIA a marked decrease in endothelin-1 immunostaining of endothelial cells was seen. Moreover, renal tubular epithelium from patients with acute allograft rejection with Banff score IB and IIA had elevated endothelin-1 immunoreactivity as compared with controls. The results showed altered endothelin-1 immunostaining in renal biopsy specimens in patients with acute transplant rejection. In acute tubulointerstitial rejection Banff IB and acute vascular rejection Banff IIA up-regulation of endothelin-1 on tubular epithelial cells was present, meanwhile in acute vascular rejection diminished immunoreactivity of endothelin-1 on endothelial cells was noted. In conclusion, our study suggests that endothelin-1 play an important role in renal tissue injury in acute tubulointerstitial and vascular allograft rejection.

Introduction

Acute allograft renal rejection (AAR) may occur within days of transplantation, or may appear suddenly months or even years later after immunosuppression has been employed. The elevation of serum creatinine level followed by clinical signs of renal failure may point to acute allograft rejection. Histological evaluation of biopsy specimens is considered one of the most valuable tools in monitoring the transplant. 

This work was supported by Medical University in Łódź, grant no 502-11-715(70)
Material and Methods

Patients

Thirty one renal allograft biopsy specimens from patients with AAR were examined by percutaneous renal biopsy. All biopsies had been performed solely for diagnostic purposes. All of our patients were adults; the mean age was 42.3±9.8 and male to female ratio was 18:13. The specimens were taken from 7 days to 5 months after engraftment (mean 49.5 days). Acute rejection was considered clinically after patients showed unexplained increase in serum creatinine concentration. Morphological diagnosis of AAR was established independently by two experienced nephropathologists according to Banff criteria [18] and based on light microscopy and immunofluorescence using standard protocols. Eleven specimens had Banff IA score: cases with interstitial infiltration (>25% of parenchyma affected) and foci of moderate intimal arteritis in at least one tubular cell. Ten specimens had IB score: cases with interstitial infiltration (>10 mononuclear cells/tubular cross section or group of 10 tubular cells). Ten specimens were classified as IIA score: cases with mild to moderate intimal arteritis in at least one arteriolar cross section. In all cases the standard immunosuppression protocol was used. As a control 10 biopsy specimens of the kidneys removed because of trauma were used (the male to female ratio was 7:3, the mean age was 38.1±7.2). None of the persons from whom renal tissue originated was known to have previous or actual renal disease. All control specimens were histologically examined by two nephropathologists and found to be normal renal tissue.

Immunohistochemistry

Paraffin sections were mounted onto superfrost slides, deparaffinized and antigen retrieval Streptavidin-biotin complex (StreptABC) technique was employed. After rehydration sections were incubated for 5 minutes with 3% hydrogen peroxide in distilled water, rinsed in Tris-buffered saline (TBS) and after blocking by normal rabbit serum (DAKO, Glostrup, Denmark) for 20min (dilution 1:5), the sections were incubated with monoclonal mouse anti-human anti-endothelin-1 antibody (clone TR.ET.48.5, Sigma, Saint Louis, USA, dilution 1:250) in a moist chamber for 1 hour at room temperature. Afterwards, sections were rinsed in TBS and incubated with biotinylated rabbit anti-mouse immunoglobulin (DAKO, Glostrup, Denmark) for 20min and StreptABComplex/HRP (DAKO, Glostrup, Denmark) for 30min prepared according to the instructions of the manufacturer. Visualisation was performed by incubating the sections in a solution of 0.5mg 3,3’-diaminobenzidine (DAKO, Glostrup, Denmark) per 1ml Tris-HCl buffer, pH 7.6, containing 0.02% hydrogen peroxide, for 10min. After washing, sections were counterstained with hematoxylin and coverslipped. Negative controls were carried out by incubation in the absence of the primary antibody and always yielded negative results.

In each specimen staining intensity of ET-1 in the endothelium of peritubular capillaries and arterioles and in the renal tubular epithelium cells were recorded by two independent observers in 7 - 10 adjacent high power fields and graded as 0 (staining not detectable), 1 (minimal immunostaining intensity in all cells) and 3 (strong staining in all cells). The mean grade was calculated by averaging grades assigned by the two authors and approximating the arithmetical mean to the nearest unity.

Statistical methods

All data are expressed as mean±SD (standard deviation). Differences between groups were tested using unpaired Student’s test preceded by evaluation of normality and Levene’s test. The Mann-Whitney U test was used where appropriate. Results were considered statistically significant if P<0.05.

Results

The mean values of immunoexpression of ET-1 are shown in Table 1.

ET-1 immunostaining in normal controls: In endothelium of peritubular capillaries and renal arterioles a strong immunoreactivity of ET-1 was seen. In epithelium of renal tubules slight to moderate immunostaining of ET-1 was detected (Fig. 1).

ET-1 immunostaining in AAR Banff IA: The mean staining intensity in endothelial cells of peritubular capillaries Arterioles Tubules

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<tr>
<th></th>
<th>Capillaries</th>
<th>Arterioles</th>
<th>Tubules</th>
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<tbody>
<tr>
<td>Controls</td>
<td>2.7±0.4</td>
<td>2.1±0.2</td>
<td>1.7±0.3</td>
</tr>
<tr>
<td>AAR Banff IA</td>
<td>2.4±0.3</td>
<td>1.9±0.3</td>
<td>1.9±0.2</td>
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<tr>
<td>AAR Banff IB</td>
<td>2.5±0.4</td>
<td>1.9±0.4</td>
<td>2.4±0.3*** (P&lt;0.001)</td>
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<tr>
<td>AAR Banff IIA</td>
<td>1.2±0.2* (P&lt;0.001)</td>
<td>0.7±0.3** (P&lt;0.001)</td>
<td>2.3±0.2*** (P&lt;0.001)</td>
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* AAR Banff IIA vs. all other groups and controls
** AAR Banff IIA vs. all other groups and controls
*** AAR Banff IB vs. AAR Banff IA and controls
**** AAR Banff IIA vs. AAR Banff IA and controls
laries and arterioles, and mean immunoreactivity of epithelial tubular cells were similar to staining intensity seen in normal controls (Fig. 2).

ET-1 immunostaining in AAR Banff IB: The mean staining immunoreactivity in endothelium of capillaries and arterioles did not differ significantly from normal kidneys and from renal tissue in AAR Banff IA. However, tubular staining was more prominent (Fig. 3) as compared with control renal tissue (P<0.001), and kidney specimens from patients with AAR Banff IA (P<0.001).

ET-1 immunostaining in AAR Banff IIA: The mean values of the endothelial immunostaining in capillaries and arterioles were significantly decreased (Fig. 4) in comparison with controls and renal tissue with AAR Banff IA and IB (P<0.001). Mean immunoreactivity of epithelial tubular cells were elevated as compared with control kidneys (P<0.001), and renal biopsy specimens in AAR Banff IA (P<0.001), however similar to staining intensity seen in AAR Banff IB (P=0.39, NS).

Discussion

Our study revealed the alterations in immunoreactivity of ET-1 in endothelium of peritubular capillaries and arterioles and epithelium of renal tubuli in AAR in transplanted kidneys. In vascular acute rejection Banff IIA there was a
significant decrease in ET-1 staining intensity in endothelial cells. In cases of severe acute tubulointerstitial rejection referred to IB Banff classification and in cases with mild to moderate vascular acute rejection Banff IIA there was increased ET-1 staining intensity in tubular epithelium. Endothelial and epithelial ET-1 immunostaining was not altered in cases with mild tubulointerstitial acute rejection Banff IA. Our results pointed to decreased endothelin-1 immunoreactivity in endothelial cells in vascular acute allograft rejection and are in concordance with others observations. Watschinger et al. [24] revealed a loss of ET immunoreactivity in intrarenal vascular endothelium in 80% of biopsies showing signs of vascular rejection, and what is more during rejection episodes plasma endothelin-1 level was elevated. These authors showed that significant reduction of ET-immunostaining in tubular epithelial cells occurred in vascular rejection, but not in interstitial rejection. In contrast, in our study elevated ET-1 immunostaining in tubular epithelium was seen in vascular rejection and in severe interstitial rejection, but in biopsies with mild interstitial rejection ET-1 staining intensity in tubular epithelial cells did not differ from control kidneys. Chareandee et al. [1] reported similar results concerning epithelial ET-1 immunoreactivity in acute allograft rejection. These authors observed elevated tubular ET-1 staining in all 18 study patients with acute

Fig. 3. Elevated ET-1 immunostaining in renal tubular epithelium in acute tubulointerstitial allograft rejection Banff IB. Magn. 400x.

Fig. 4. Decreased ET-1 immunostaining in endothelium of peritubular capillaries in renal biopsy specimen in acute vascular rejection Banff IIA. Magn. 400x.
Endothelin-1 in renal allograft rejection

rejection and in 5 out of 7 patients with chronic rejection. Unfortunately, their observations referred to acute allograft rejection without distinguishing between interstitial or vascular rejection. It is also worthy of note that in all previous ET-1 immunostaining study in renal biopsies from patients with renal allograft rejection the authors did not referred intensity of ET-1 immunoreactivity in respect to Banff classification. Thus, it may be the reason for the divergent results. It is obvious, that the intensity of renal tissue damage varies between IA and IB Banff allograft rejection score. In Banff IA only mild tubulointerstitial changes are seen, while in Banff IB severe tubulointerstitial damage is noted. In our study cases with vascular acute rejection referred to Banff IIA (mild to moderate intimal arteritis); but we did not evaluated ET-1 immunoreactivity in cases with severe arteritis or cases with transmural arteritis and arterial fibrinoid change or necrosis of medial smooth muscle cells. What is more, the differences in tissue processing and antibodies used may also reflect differences between our results and others. It is well known that endothelin is an important mediator of pathophysiological alterations in renal hemodynamics. ET actions in the kidney are mediated primarily by paracrine and autocrine mechanisms [9]. In normal human kidney renal vessels are an important source of intrarenal ET-1 secretion [5], however, several studies documented ET-1 synthesis by renal tubular epithelium [5, 21]. Experimental studies in animals showed that ET-1 helps control the reabsorption of sodium in proximal tubules and water in cortical collecting ducts [5, 21]. Tubular ET-1 has also been proposed to mediate tubulointerstitial injury by regulating cell proliferation, matrix accumulation, and regional blood flow [4, 16]. Release of ET-1 from endothelial cells is stimulated by many vasoactive agents such as bradykinin, angiotensin II, vasopressin and inflammatory agents - interleukin-1, tumor growth factor-beta, tumor necrosis factor, thrombin [6, 10, 25, 26]. It is obvious, that renal vasculature and renal tubules are important site of injury in acute allograft rejection of transplanted kidneys. Endothelium constitutes one of the main targets for the infiltrating leukocytes, moreover severe pathological changes including swelling, necrosis, thrombosis, platelet and fibrin aggregation are indicative of direct endothelial damage. On the other hand, lymphocytic infiltration of tubular epithelium (tubulitis) and tubular damage are demonstrated in all patients with acute interstitial rejection.

In summary, our results revealed altered ET-1 immunoreactivity in endothelial and tubular epithelial cells in Banff IB tubulointerstitial acute allograft rejection and Banff IIA vascular acute allograft rejection, however whether these findings may account for tissue damage and clinical signs observed during acute allograft renal rejection needs to be further studied.

References


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