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Original Article

Granzyme B and TIA-1 Expression in Chronic and Acute on Chronic Renal Allograft Rejection

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Although active inflammation may be deleterious and indicate immunologic activation in chronically rejected grafts, the underlying mechanism of tissue destruction has been little studied. Twenty-four cases of chronic rejection (CR) with or without acute rejection (AR) were stained with antibodies against CD3, CD8, CD68, granzyme B and TIA-1, and the number of positive cells were counted. Eleven cases of AR served as controls. The number of CD3 and CD8 positive cells increased in the acute on CR group compared to the CR group. About a half of CD3 positive T cells were CD8 positive in both groups, however, the proportion of TIA-1 or granzyme B positive cells was higher in the acute on CR group. The numbers of CD3, CD68, granzyme B and TIA-1 positive cells were higher in the AR group than the acute on CR group, however, no significant difference was found between the two groups. Serum creatinine level and proteinuria at the time of biopsy and the percentages of late onset AR and graft failure rate were higher in the acute on CR group than the CR group. Summarizing, these results suggest that infiltration of activated T cells containing cytotoxic granules plays a role in graft destruction in acute on CR.

Key Words: Acute on chronic rejection, chronic rejection, cytotoxic cells, granzyme B, renal transplantation, TIA-1

INTRODUCTION

Chronic rejection (CR) is the most common cause of chronic renal allograft dysfunction. CR is defined clinicopathologically by a slow increase of serum creatinine and combined features of glomerulosclerosis, tubular atrophy, interstitial fibrosis and vascular intimal thickening.[1-3] An episode of acute rejection (AR) is one of the most important determinators related to the development of or progression to CR, especially with respect to its incidence and time of onset after transplantation.[4-6] In AR, renal allografts are destroyed by inflammatory infiltrates in the tubules and interstitium, and by vasculitis. T cells, especially activated cytotoxic T cells, are known to have a major role in acute graft destruction by delivering the death signal by Fas-FasL, the formation of pores in target cell membrane or release of cytotoxic granules,[7,8] such as granzyme[9,10] and TIA-1.[11,12] Although progressive fibrosis is a dominant finding in CR, active inflammation caused by immune-activation may also participate in destruction of grafts.[13] The mechanism of tissue destruction in this acute on CR, however, has been little studied. Ode-Hakim et al.[14] reported an upregulation of IFN-gamma, IL-4, IL-10, and TNF-alpha transcripts but no elevation of granzyme A transcripts in peripheral mononuclear cells and graft infiltrating cells in late AR of renal allografts, and suggested delayed-type hypersensitivity-like mechanisms. Sharma et al.[15] studied expression of FasL mRNA and granzyme B in renal allograft rejection, using renal tissue homogenate. Expressions of both were increased in AR but not in CR or acute on CR. However, Barth et al.[16] reported that granzyme expression in urine lymphocytes was increased in early CR, suggesting a constant cytotoxic activity. The cause of this discrepancy of the results is not unknown, however, it may be attributed to the material and methods used in the studies. Inflammatory process may be variable from area to area, therefore, tissue for molecular study may not have the similar degree of inflammation to that for routine histology.

To investigate the role of cytotoxic cells in

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chronic renal allograft dysfunction, the expressions of granzyme and TIA-1 were examined in the tissue sections of CR and acute on CR.

MATERIALS AND METHODS

Materials

From a biopsy file, each twelve cases of CR and acute on CR were consecutively selected. The patients received cyclosporine and prednisolone based immunosuppression. The criteria for CR was defined according to Banff schema.[17] Acute on CR was defined as the presence of CR and the presence of tubulitis in non-atrophic tubules and mononuclear cell infiltrates associated with edema in the non- fibrotic interstitium (Fig. 1). Medical records were reviewed for the following parameters: age and sex of patients, HLA match, number and time of onset of clinical AR, duration from transplantation to biopsy, 24-hour proteinuria and serum creatinine level at the time of biopsy.

Histologic studies

For light microscopy, renal cores were formalin-fixed, paraffin-embedded, sectioned at 2-3 ㎛ and stained with hematoxylin-eosin, periodic acid-Schiff, acid fuchsin orange G and periodic acid-methenamine silver. Stains for CD3 (Dako, Glostrup, Denmark, dilution 1:100), CD8 (Dako, dilution 1:50), CD68 (Dako, dilution 1:75), granzyme B (Monosan, Front straat 2a, 5465 PB Uden, Netherlands, dilution 1:40) and TIA-1 (Beckman Coulter, Brea, CA, USA, dilution 1:2,000), were performed on paraffin-embedded 2-3 ㎛-thick renal sections, using the LSAB kit (Dako). Substrate reaction was performed using AEC (3-amino-9-ethylcarbazole) and the sections were counter-stained with Mayer hematoxylin. Eleven cases of AR served as controls. The number of positively stained cells were counted at × 400 magnification at cortical interstitium and the result was expressed as mean ± SD/mm^2. Comparisons between groups were performed using Mann Whitney non-parametric test. A p-value less than 0.05 was considered significant.

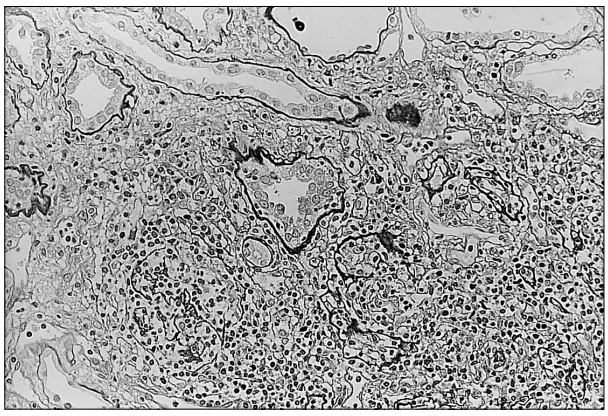

Fig. 1. The photomicrograph depicts interstitial mononuclear cells infiltration and non-atrophic and atrophic tubules with tubulitis and basement membrane destruction in acute on chronic rejection (PAS).

RESULTS

Clinical findings

The number of episodes of clinical AR were 0.6 in the CR group and 2.1 in the acute on CR group before biopsy. A history of clinical late AR (one year after renal transplantation) occurred in one case of the CR group and 8 cases of the acute on CR group. Five cases of the acute on CR group had biopsy-proven acute tubulointerstitial rejection episodes previously. Serum creatinine level and proteinuria at the time of biopsy were higher in the acute on CR group than in the CR group, and statistically significant in the former (Table 1).

Immunohistochemistry

CD3 and CD68 positive cells were predominantly infiltrating cells in CR and were present diffusely or in small clusters in the interstitium (Fig. 2). TIA-1 and granzyme B positive cells were scattered in the interstitium, tubules and rarely in the glomeruli (Fig. 3). Both the numbers of CD3 and CD8 positive cells and TIA-1 or granzyme positive cells were higher in the acute on CR group than in the CR group. About a half of CD3 positive cells were CD8 positive in both groups, however, the proportion of TIA-1 or granzyme

Table 1. Clinical Findings

Croups	CR	Acute on CR	p value
No. of cases	12	12	
Age / Sex (M:F)	34.3 ± 7.1(8:4)	26.5 ± 9.0(9:4)	0.029
Duration from Tx to Bx (month)	53.7 ± 21.6	34.0 ± 22.2	0.039
(range)	(14 - 84)	(11 - 69)	
No. of cases with clinical AR			
Within 1 yr	6(50.0%)	8(61.5%)	
After 1 yr	1(8.3%)	8(61.5%)	
Serum creatinine at time of Bx (mg/dl)	2.2 ± 1.1	4.0 ± 1.9	0.010
Proteinuria at time of Bx (g/24 h)	1.4 ± 1.4	2.8 ± 2.8	0.130
Type of allografts living related/unrelated	4/ 8	1/ 12	
HLA match			
A+B	1.5 ± 0.5	1.3 ± 0.8	0.669
DR	1.0 ± 0.0	1.1 ± 0.3	0.350

Tx, transplantation; Bx, biopsy; CR, chronic rejection; AR, acute rejection.

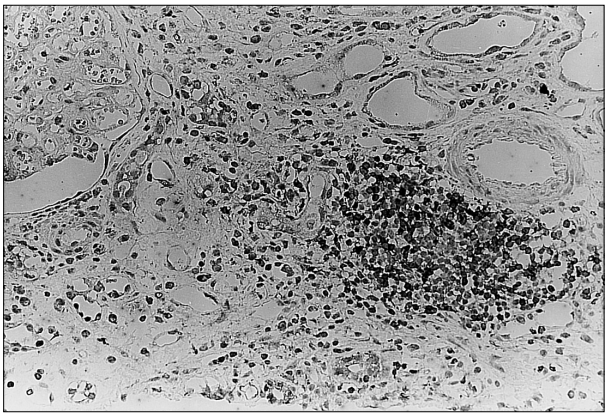

Fig. 2. CD3 positive cells are scattered or in small clusters in the interstitium in acute on chronic rejection.

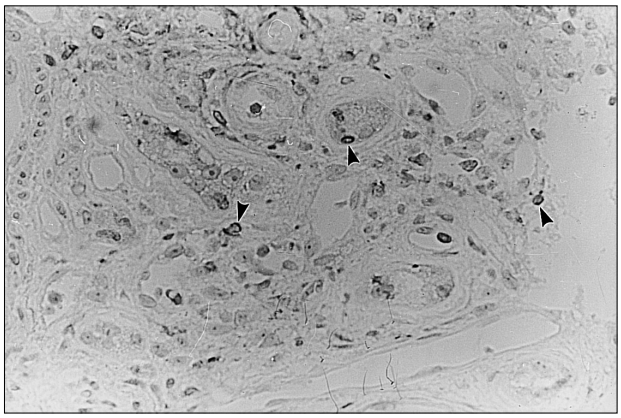

Fig. 3. Granzyme B positive cells are scattered in the interstitium and between tubular epithelial cells (arrows) (same case as in Fig. 2).

positive B cells were higher in the acute on CR group. One case of the CR group showed minimal increase of granzyme positive cells, whereas 4 cases of the acute on CR group did not show granzyme positivity. Two cases of the CR group and 8 cases of the acute on CR group showed infiltration of TIA-1 positive cells in the interstitium. The numbers of CD3, CD8, CD68, TIA-1 and granzyme B positive cells in the acute on CR and AR groups were not significantly different (Table 2).

Follow-up

Four cases of the CR group and two cases of the acute on CR group underwent repeat biopsies, due to further creatinine elevation. In addition to the persistence or progression of tubulointerstitial fibrosis, three cases showed cyclosporine nephrotoxicity, and each one case developed mem

Table 2. Immunohistochemical Findings

Groups	AR	CR	A-CR	p value		
				AR vs CR	AR vs A-CR	CR vs A-CR
CD 3	686.2±343.0*	245.3±201.3	504.5±248.6	0.001	0.166	0.010
CD 8	248.0±118.9	123.3±97.6	267.1±150.4	0.014	0.749	0.011
CD-68	721.7±461.9	334.7±217.4	581.9±505.6	0.016	0.498	0.134
TIA-1	83.5±50.1	8.0±18.5	45.6±49.3	0.000	0.082	0.002
Granzyme B	58.6±50.5	1.4±4.1	25.3±23.8	0.001	0.055	0.022

AR, acute rejection; CR, chronic rejection; A-CR, Acute on chronic rejection.
*number of positive cells/mm^2 cortical interstitium.

branous nephropathy and IgA nephropathy. Grafts failed in five cases of the CR group and nine cases of the acute on CR group during an average follow-up of 34.8 and 26.1 months after biopsy. Graft failure rate was higher in cases with a history of AR one year after transplantation than without (77.8% versus 46.7%).

DISCUSSION

Although much advance has been made in the understanding of the pathogenesis of AR,[18-20] the destruction mechanism of chronically rejected grafts has been little studied. The degrees of tubular atrophy and interstitial fibrosis are major histologic determinants for graft failure in CR, and an increased expression of TGF-β by inflammatory cells and fibroblasts is believed to be important.[21] Generally, graft function deteriorates progressively and slowly in CR. However, clinical course may be variable from patients to patients,[22] which suggests an involvement of other mechanisms in graft destruction. These may be the involvement of nonimmunologic factors, inadequate immunosuppression, etc. Repeated or late AR episodes are a well known predictor for CR, which suggests a role of active inflammation in graft destruction. In this study, we have demonstrated an involvement of cytotoxic mechanisms in acute on CR.

The number and ratio of granzyme B and TIA-1 positive cells were higher in the acute on CR or AR group than in the CR group. This increased cytotoxic activity in the acute on CR group may explain the more frequent episodes of AR or late onset AR, and the increased graft failure rate in the study. Since no difference was observed between the AR and acute on CR groups in terms of granzyme B or TIA-1 positivity, histologic features of acute on CR may represent late AR at least in some cases, clinically. Contrary to the report that perfect HLA matching decreased both early and late AR rates,[23] no significant difference was found in the degrees of HLA match between the CR and acute on CR groups in our study. The number of granzyme B positive cells were less than the number of TIA-1 positive cells. We think granzyme B positivity to be more reliable than TIA-1 positivity for the demonstration of cytotoxicity, since TIA-1 positivity tended to be variable from case to case. The expression of granzyme B and TIA-1 may be helpful in predicting inflammatory activity in subclinical rejection,[24] which has a controversy in treatment and impact on the progression to CR.[25,26] CD68 positive macrophage infiltrates were not significantly different between the CR and acute on CR groups. Although macrophage subpopulation may be important in this setting,[27,28] it was beyond the scope of this study.

Summarizing, cytotoxic mechanism was present in renal acute on CR cases. The presence of active inflammation is important since it can accelerate the speed of destruction in grafts with already reduced functioning parenchyma.

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