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Retrospective study of histological features of acute rejection in renal allografts and comparison with circulating T cell populations

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SUMMARY The histological severity of acute rejection in renal allografts was determined for 39 rejection episodes in 30 renal transplant recipients. Data were compared with the peripheral blood T cell subset ratios measured before and at the onset of the rejection episode. T cell subset ratios showed no correlation with the histological severity of rejection, nor with the reversibility of the rejection episode. The grade of histological rejection on biopsy was predictive of graft survival. We conclude that renal biopsy remains the best method for determining the severity and outcome of acute allograft rejection episodes.

Renal biopsy has an established role in the diagnosis of rejection in renal transplant recipients. The histological severity of rejection predicts the response of the rejection episode to treatment and may have prognostic significance for long term graft function.[1–4]

Changes in circulating lymphocyte populations in transplant recipients were first described in 1976,[5] and, more recently, monoclonal antibodies to specific lymphocyte subsets have been used to monitor their fluctuations during rejection. A consistent pattern of change may provide a rapid diagnostic test for rejection. Several workers have suggested that the ratio of helper to suppressor/cytotoxic lymphocytes increases before the onset of rejection and falls as the process is reversed.[6–9] Other centres, however, have been unable to confirm this relation.[10–13]

The aims of this study were to examine the relation between the histological changes in rejecting renal allografts and measurements of peripheral blood T cell subsets before and during rejection. The prognostic value of the two were compared.

Patients and methods

Data on 30 recipients of renal transplants performed in the Regional Transplant Centre at St James's Hospital, Leeds, between September 1982 and September 1983 were studied. All patients experienced acute rejection episodes, during which peripheral blood T cell subset measurements were made. There were 21 men and nine women, with a mean age of 35·4 (range 15–60) years. All grafts were of cadaveric origin. Standard immunosuppression with steroids and azathioprine was used according to a previously described regimen.[14] Acute rejection episodes were treated with increased doses of corticosteroids, up to 5 g methyl prednisolone over five days. The date of onset of rejection was taken as the day of first increase in a previously falling or stable serum creatinine concentration, accompanied by clinical signs of rejection. A clear date of onset was defined by these criteria in 23 of 39 rejection episodes. The follow up interval for graft survival is between 16 and 28 months.

HISTOLOGICAL STUDIES

Material available for histology consisted of open wedge biopsy (30) and nephrectomy specimens (9) obtained during the rejection episode. More than one specimen was received from eight patients; these were regarded as separate rejection events for the purposes of this study. At least five sections were studied in each case. The recently described method of Matas *et al*[1] was slightly modified and used to assess the histological severity of acute rejection.

This method categorises separately the degree of vascular rejection and intensity of tubulointerstitial inflammation. Acute vascular rejection is characterised by endothelial swelling and infiltration by mononuclear cells; when this change was seen in more than half the arteries and arterioles in the

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biopsy it was graded as moderate (++), and when in less than half as mild (+). Severe (+++) acute vascular rejection was diagnosed when any of the vessels showed necrotising vasculitis, fibrinoid necrosis, or fibrin-platelet thrombosis.

Acute tubulointerstitial rejection shows the features of an interstitial mononuclear infiltrate, interstitial oedema, and tubular damage. This was graded on a scale of mild (+) to severe (+++), depending on the intensity of inflammation and whether the changes were focal (+) or diffuse (+++).

This method also includes a system for grading chronic vascular and chronic tubulointerstitial rejection, but as these are not relevant to the present study they are not described here.

The degree of rejection was determined for all wedge biopsies without knowledge of the clinical outcome, and all specimens were assigned a separate grade of acute vascular and acute tubulointerstitial rejection.

IMMUNOLOGICAL STUDIES

Peripheral blood T lymphocytes had been estimated in these patients three times a week during their stay in hospital and weekly after discharge. Blood samples from patients in hospital were all obtained between 9 and 10 am. Lymphocytes were isolated from heparinised blood on a Ficoll-Hypaque gradient, washed, and stained with commercially available monoclonal antibodies (Orthoclone) to total mature T cells (OKT3), helper T cells (OKT4), and suppressor/cytotoxic T cells (OKT8), according to a previously described technique.[15] The ratio T4:T8 was derived and used for comparison with histological data because this ratio is claimed to be a more reliable indicator of rejection than absolute T cell numbers.[8]

The subset ratios in blood samples, collected within a day of biopsy or nephrectomy, were available in 26 cases, and samples from four to six days previously were available in 29 cases.

Results

HISTOLOGY

Histological evidence of acute rejection was present in all specimens. Two of the biopsies (7·4%) contained fewer than four vessels and were therefore inadequate for quantification of acute vascular rejection. These were assigned to acute tubulointerstitial rejection grades only. Table 1 shows the number of cases in each diagnostic group.

Biopsy or nephrectomy was performed at variable times (1–10 days) after the onset of rejection. Fig. 1 shows the relation between the histological grade (combined acute vascular and acute tubulointerstitial rejection) and the time between the first rise in creatinine and biopsy or nephrectomy for those patients in whom the date of onset of rejection could be reliably determined. There was no tendency for later samples to show greater severity, which suggests that the severity of a given rejection episode remains constant and is mild or severe from the outset.

Twenty of the biopsies were obtained either during treatment for acute rejection or less than six days after finishing treatment. A further 10 were obtained at least seven days after completing acute antirejection treatment. Table 2 shows the relation with histological grade. The presence of severe histological rejection during a course of steroids implies that they did not modify the morphological expression of rejection in those patients. In addition, the prognostic value of biopsy was not affected by concurrent steroid treatment for the rejection episode.

IMMUNOLOGY

Immunofluorescence using the OKT series of monoclonal antibodies gave measurements of T4 positive and T8 positive cells, which agreed with the total percentage of T3 positive cells in most cases. In five instances (8% of measurements) the sum of T4 plus T8 positive cells was significantly higher (>15% higher) than the total number of T3 positive cells, which implies the presence of phenotypically atypical T cells (T4 and T8 positive or T3 negative cells). In these cases the T4:T8 ratio was invalidated and not used for comparison with the histological appearances of the graft. This phenomenon was not consistently associated with high dosage steroid treatment. T4:T8 ratios fluctuated considerably in individual patients after transplantion. No consistent pattern was seen before or during a rejection episode, nor did the use of steroids have a predictable effect on the subset ratios during such an episode.

COMPARISON OF HISTOLOGICAL APPEARANCES WITH T CELL SUBSET RATIOS

T cell subset ratios estimated within a day of biopsy or nephrectomy in 26 cases were compared with the severity of acute vascular and acute tubulointerstitial rejection (Fig. 2); the circulating T cell subset ratios bore no relation to the intensity of the rejection process within the graft, as seen histologically.

In view of reports that circulating T cell subset changes may preceed the clinical appearance of rejection by several days, we compared the T cell subsets measured four to six days before biopsy or nephrectomy (29 cases) with the histological severity of acute vascular and acute tubulointerstitial

Table 1 *No of cases with each histological grade of rejection*

	Grade of rejection			
	–	+	++	+++
Acute vascular rejection	5	14	9	9
Acute tubulointerstitial rejection	1	11	12	15
	Mild (1–2+)	*Moderate (3–4+)*	*Severe (5–6+)*	
Combined (acute vascular and acute tubulointerstitial rejection)	13	15	11	

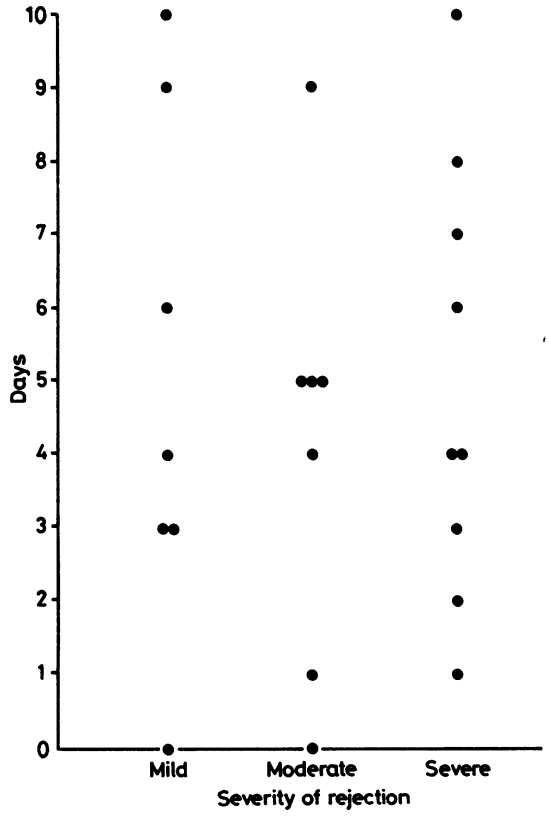

Fig. 1 *Severity of rejection v number of days between onset of rejection and biopsy or nephrectomy.*

rejection (Fig. 3). Circulating T cell subsets did not forecast the intensity of histological rejection.

PROGNOSTIC VALUE

The prognostic value of biopsy during rejection is established, and our findings relating the outcome of the transplant to the severity of rejection seen on biopsy are comparable with those of other authors[1] (Table 3). In five patients there were two biopsies showing different degrees of rejection, but only the more severe grade is included in Table 3. One patient with severe rejection on biopsy had good long term function. She was later found on isotope scanning to have an area of infarction in an otherwise functioning graft. The other four biopsies showing severe rejection were followed by nephrectomy, after an average of three weeks (range one to eight weeks). The four nephrectomies following mild or moderate acute rejection biopsies were performed for chronic rejection (three) or cytomegalovirus infection (one). Morphological features of cytomegalovirus infection could not be identified in this kidney. Eleven patients with mild or moderate rejection on biopsy had functioning grafts after 16–28 months. In two of these patients with moderate rejection there was chronic impairment of graft function with a serum creatinine concentration greater than 200 μmol/l.

T cell subset ratios estimated before transplantation, four to six days before a rejection episode, or at the onset of a rejection episode (Fig. 4) showed no prognostic value with regard to either reversibility of the rejection episodes or long term graft survival.

Table 2 *Histological grade of rejection v steroid treatment of rejection episode*

Grade of rejection	*Steroids <6 days before biopsy*	*No steroids for >6 days before biopsy*
Mild	6	3
Moderate	5	5
Severe	9	2

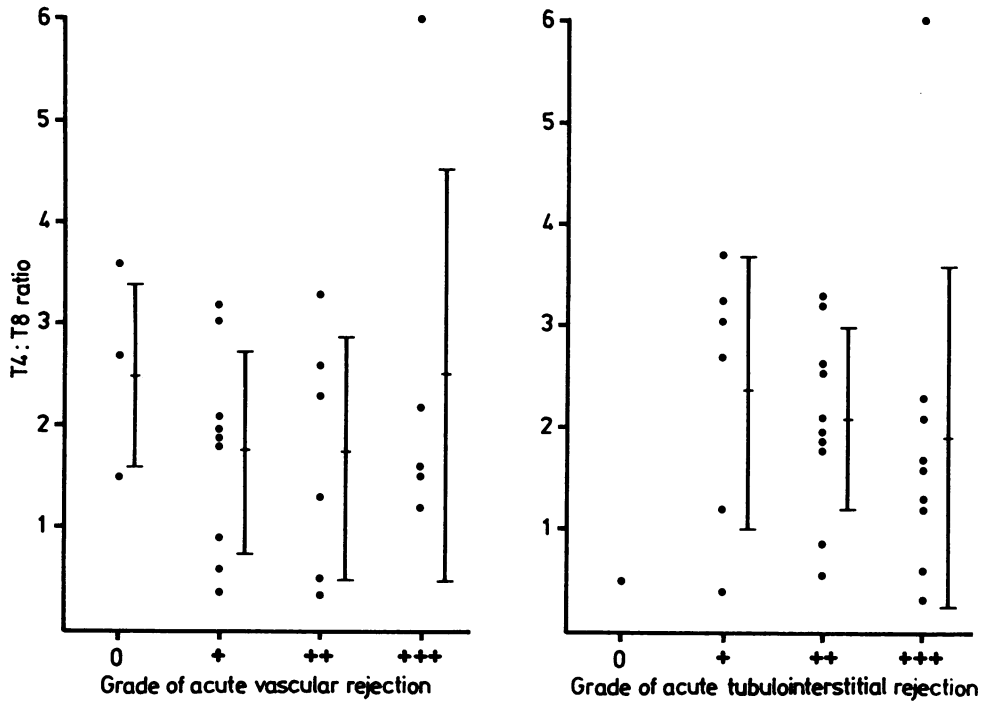

Fig. 2 *T4:T8 ratio measured within one day of biopsy or nephrectomy v histological grade of rejection. Bar shows mean* (± 1 SD).

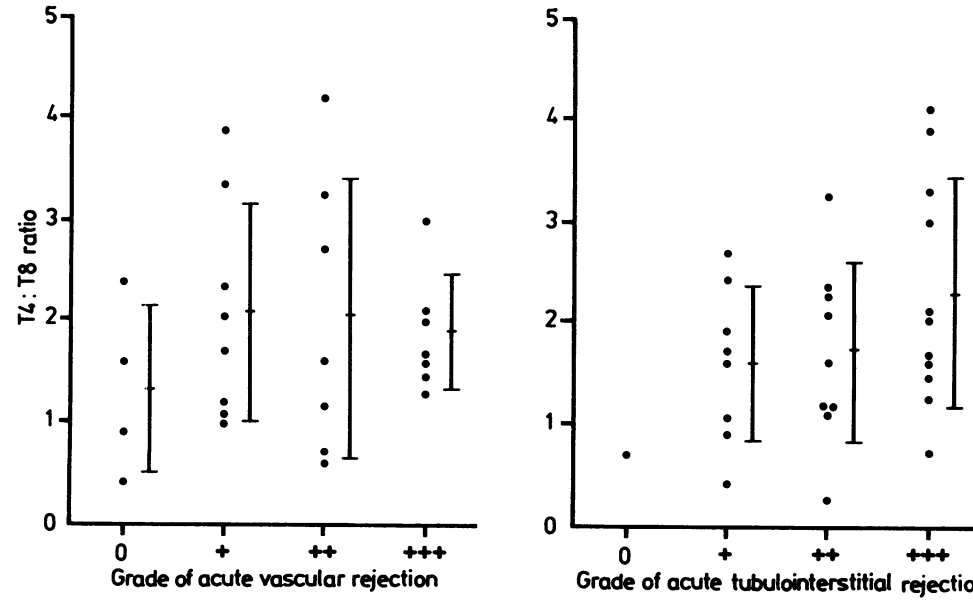

Fig. 3 *T4:T8 ratio measured four to six days before biopsy or nephrectomy v histological grade of rejection. Bar shows mean* (±1 SD).

Table 3 *Outcome of transplant v histological grade of rejection on biopsy in 22 patients*

	Severity of rejection		
	Mild	*Moderate*	*Severe*
Good allograft function	4	5	1
Chronic impairment of function		2	
Nephrectomy	1	3	4
Death of patient	1	1	

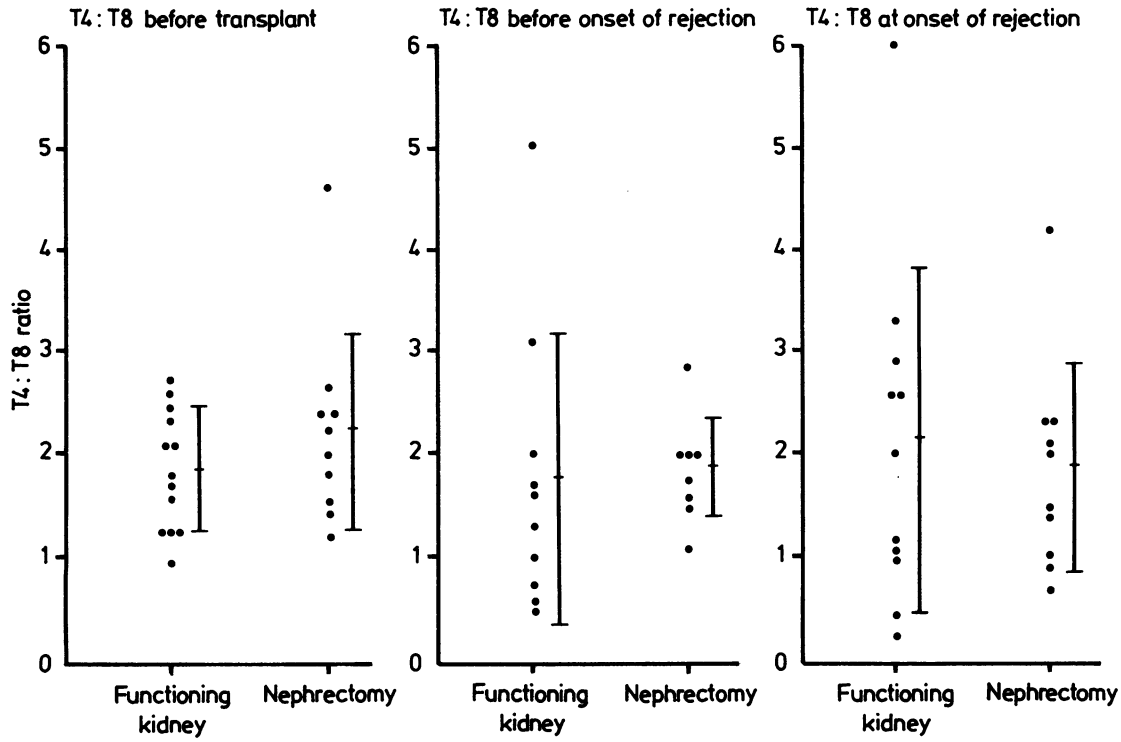

Fig. 4 *Ratio of T4:T8 v outcome of transplant.*

Discussion

This study was confined to those patients undergoing rejection episodes in the early post-transplant period for whom histological documentation of rejection was available. Previous studies concerning T cell subset monitoring have used clinical criteria for the diagnosis of rejection episodes but histological confirmation was not always available.[6 9–12 16]

We were unable to find any correlation between circulating T cell subset ratios and the occurrence of histologically proved rejection; in addition, no consistent relation between subset data and the vascular and tubulointerstitial components of rejection was apparent when these variables were considered separately.

Several authors have claimed that the T4:T8 ratio rises during rejection episodes[6–9 17] or increases before rejection, or both.[7 16] In contrast, others have found the ratio to be unchanged during rejection.[10–13] Claims have been made that this ratio predicts the occurrence of rejection[6 16] or its reversibility[16] and pretransplant ratios may even predict ultimate graft loss from rejection.[13 16] These assertions have not been confirmed by other centres.[9 11 12] It has been suggested that such inconsistency may be due partly to the diversity of immunosuppressive regimens used by different centres.[12] In our experience with conventional immunosuppression the T cell subset ratios fluctuated widely both within patients and between different patients, irrespective of clinical events in the post-transplant period.

Neither absolute numbers nor T cell ratios could be correlated with rejection in any way.

Characterisation of mononuclear cells infiltrating the renal tissue within rejecting allografts has consistently shown a high proportion of suppressor/cytotoxic (T8 positive) cells, with an inverse ratio of T4:T8 relative to that present in the circulation.[18-20] Again, however, there is lack of agreement over the correlation of T cell status within the graft with the severity or reversibility of rejection. It is not known whether the population of T8 positive cells in the graft consists predominantly of cytotoxic or suppressor cells, nor do we know if these are the same as the T8 positive lymphocyte population in the peripheral blood. There seems, therefore, to be no reason to expect the peripheral blood subsets to mirror events occurring within the rejecting graft. Histological appearances reflect the degree of renal damage resulting from the sum of various immunological mechanisms operating during rejection, of which the T4:T8 ratio is at best merely one variable.

No information is available regarding the histological changes occurring in the allograft during the evolution of a rejection episode, especially with regard to the influence of treatment on these appearances. Our studies suggest that the severity of rejection remains constant during a given episode and that the histological appearance is unaffected by concurrent antirejection steroid treatment.

We conclude that renal biopsy remains the best method for determining allograft rejection and is efficient in predicting graft survival. The significance of the histological features appears to be unaffected by the timing of the biopsy or the concurrent treatment of the rejection episode. Conversely, we have found peripheral blood T cell monitoring a time consuming and highly expensive exercise which has not aided clinical management.

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