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Original Article

The Diagnostic Value of Detection of CD20 Positive Infiltrates in Renal Biopsies with Acute Allograft Rejection: A pilot study

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Abstract

Introduction: The recognition of antibody mediated rejection has led to re-appreciation of the role of B cells in acute and chronic allograft rejection. The presence of CD20 positive lymphocytic infiltrates in acute cellular rejection has been associated with poor clinical outcomes and reduced graft survival. Recently molecular gene analysis has shown that grafts with antibody-mediated rejection (ABMR) have lower expression of CD20.

Methods: We reviewed 28 renal allograft biopsies, including 13 biopsies from patients who experienced acute ABMR and a matched group of 15 patients with acute T cell mediated rejection (TCMR) to serve as controls. All biopsies were stained by anti-CD20 and anti-CD8 antibodies.

Results: All twenty-eight biopsies were found to have CD20 positive cells within their interstitial infiltrate. The distribution of CD20 positive cells varied from sparse cells to small or dense clusters in the interstitium. We found no statistically significant differences in CD20 or CD8 cell counts between the ABMR and TCMR groups. We noticed a weak positive correlation between the numbers of CD20 positive cells and the grade/severity of rejection but it didn't reach statistical significance ($r=0.37$, $p=0.06$). However, we found a significant positive correlation between the number of CD20 positive cells and intimal artertitis score ($r=0.39$, $p<0.05$).

Conclusion: Our findings suggest that there is a possible relation between the presence of CD20 positive lymphocytic infiltrates and a more severe histological form of rejection. However, we failed to establish a relationship between their actual presence in the interstitial infiltrate and distinct mechanisms of graft rejection.

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Introduction

Acute allograft rejection represents an important complication after transplantation with significant impact on long-term graft survival. The distinction between cellular rejection and humoral rejection is a continuous challenge in clinical transplant immunology.

The diagnosis of T cell mediated cellular rejection (TCMR) is based on the histological finding of lymphocytic infiltrates, primarily CD4+ve and to a lesser extent CD8+ve T cells in the tubulointerstitial space with demonstrable tubulitis [1, 2]. On the other hand, humoral rejection, now termed antibody-mediated rejection (ABMR), is thought to be a consequence of allospecific antibodies produced by B lymphocytes demonstrable by the presence of C4d peritubular capillary deposition and circulating donor-specific antibodies in addition to evidence of microvascular injury in the tissue [3].

The involvement and relevance of B lymphocytes in either process is still not clear. CD20 is a cell-surface marker unique to B-lymphocytes. However, CD20 expression is lost during differentiation into antibody-secreting plasma cells [4]. Recent interest in the presence of CD20-positive B-lymphocytes in the renal interstitium of patients with acute cellular rejection was gained by the work of Sarwal and collegues[5]. Utilizing immunohistochemical staining, they found that most of patients who expressed genomic signatures consistent with the presence of B-lymphocytes also demonstrated clusters of CD20 positive lymphocytes in the allograft interstitium and that these infiltrates correlated with steroid-resistant rejection and worse graft outcomes.

This was followed by another study by Benjamin *et al* who confirmed an association between the presence of CD20 positive lymphocytic infiltrates in acute cellular rejection and poorer clinical outcomes, including reduced graft survival [6]. Other reports included a novel form of monocyte predominant rejection associated with Campath 1-H therapy with incidentally observed CD20 positive B cell clusters in patients with acute allograft rejection, and the presence of nodular CD20 infiltrates in biopsies with acute rejection [7, 8].

A recent report of molecular phenotypes of acute rejection showed that grafts with ABMR had lower expression of CD20. The authors reported that B cell infiltration (CD20) was higher in TCMR than in ABMR and suggested that one of the strongest prediction of graft failure within 12 month is defined by CD20 gene expression levels in the biopsy, revealing early ABMR episodes [9].Conversely, other studies showed no correlation between CD20 positive infiltrates and patient's outcome [10, 11].

The recognition of antibody mediated rejection, the availability of anti-CD20 monoclonal antibody (Rituximab) and the publication of isolated case reports showing the efficacy of Rituximab in therapy of resistant allograft rejection have led to re-appreciation of the role of B cells in acute and chronic allograft rejection [12].

Methods

We retrospectively reviewed 90 renal allograft biopsies received from different transplant centers in the period from 2010 to 2011. We defined a very narrow subgroup according to preset selection criteria. Out of the 90 patients reviewed, 13 patients who experienced acute ABMR episodes (C4d+ve group) were included. We also identified a matched group of 15 patients diagnosed as Banff type I/II cell mediated rejection according to Banff 1997 classification criteria (C4d-ve group) to serve as controls [3].

Patients were included in the study according to the following criteria:

1. Biopsy proven acute rejection (ABMR or TCMR) within first year post transplant (since rejection episodes occurring beyond the first year post transplant are more likely to have a mixed histological picture.)

2. Adequate biopsy according to the Banff classification adequacy criteria.

3. Availability of preliminary clinical data (time post-transplant, immunosuppressive therapy and demographic data of the patients) in the pathology report.

4. Presence of enough remnant tissue to perform immunohistochemistry studies.

Patients were excluded if:

1. The biopsy was inadequate according to the Banff classification adequacy criteria

2. Biopsies showed chronic tubulointerstitial changes graded as > IFTA grade I (ci:1 and ct:1).

3. Pre-biopsy treatment with anti-rejection therapy.

4. Incomplete clinical information.

5. No available tissue material for further studies.

6. Or if there were any associated conditions such as recurrent disease, discontinuation of medication due to infections or post-transplant lymphoproliferative disorder, and graft loss due to technical reasons.

From each case existing unstained slides or newly prepared slides were stained with Hematoxylin and Eosin, Masson trichrome stain and Periodic acid Schiff for routine histopathological examination. Cases were reviewed and scored according to the Banff grading system for acute and chronic changes [3].

Charged slides were treated for CD20 and CD8 antibodies (Dako, Carpinteria, CA), (HRP/DAB, Lab vision, USA). Paraffin-embedded tissue sections of lymph nodes were used as positive controls for CD20. Negative controls were obtained by incubating serial sections with the blocking solution and then omitting the primary antibody.

Entire cores were scanned to determine the density of CD20+ve and CD8+ve cells and the number of positive cells were counted in 10 randomly selected high-power fields (HPF; ×400) in the cortical region. Cell density per high power field and the number of high power fields counted per core were documented. The results were expressed as a mean number of immune-positive cells per biopsy.

C4d (Springbio, USA) staining was evaluated for the percent of peritubular capillaries (PTC) with circumferential and linear basement membrane staining pattern in either cortex or medulla, excluding scarred or necrotic areas as recommended by the Banff classification. C4d was defined as positive when at least 10 adjacent peritubular capillaries were positive.

Dichotomous variables were analyzed with the chi-square test and continuous variables with correlation analysis to study the relation between CD20, CD8 and C4d positivity and the relation between cell counts and pathological parameters in both study groups respectively. A p-value of < 0.05 was considered statistically significant.

Figure 1: (A) Histopathological section displaying large clusters of CD20 positive B cells; (B) Histopathological section displaying CD8 positive cells; (C) Diffuse C4d positivity in peritubular capillaries

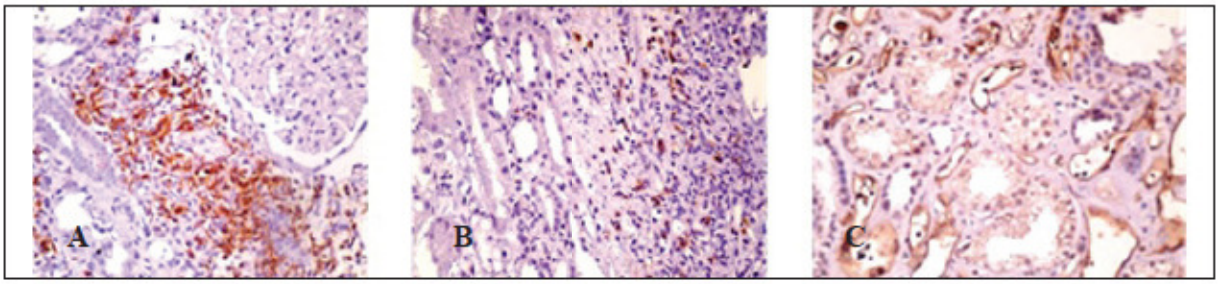


Table 1: Mean cell counts of CD20 and CD8 positive cells in the study groups

	C4d -ve	C4d +ve	P value
CD20	23 ±10	18 ±9	0.13
CD8	22 ±11	17 ±9	0.24

Results

The study included biopsies from 28 patients, 17 males (61%) and 11 females (39%) with a mean age of 34±10 years and mean serum creatinine of 3.6±1.5 mg/dl (range 1.4-7.0 mg/dl). Patients' age and sex were not different between the two groups and did not correlate with any of the histological parameters, C4d, CD20 or CD8 cell counts.

All twenty-eight biopsies were found to have CD20 positive cells within their interstitial infiltrate. The distribution of CD20 positive cells varied from sparse cells to small or dense clusters in the interstitium (Figure-1). There was no evidence of follicular formation in any of the CD20 positive clusters. There was no statistically significant difference in CD20 and CD8 cell counts between the C4d negative and C4d positive groups (Table-1).

On analyzing the individual scores of acute rejection, we found a significant positive correlation between the number of CD20 positive cells and the intimal arteritis (v score) signaling acute vascular rejection ($r=0.39$, $p<0.05$) (Figure-2). No correlation was found between CD20 cell counts and the rest of the acute scores (t, i, and g). CD8 cell counts didn't correlate with any of the acute scores.

There was no relation between the C4d positivity and the degree of interstitial inflammation (i score), intimal arteritis (v score) or glomerulitis (g score). However, there was a negative correlation between C4d positivity and the grade of tubulitis (t score), ($r= -0.39$, $p= 0.04$) (Figure-3).

A marginal positive correlation between the numbers of CD20 positive cells and C4d positivity was observed but it didn't reach statistical significance ($r=0.34$, $p=0.07$). No correlation between CD8 cells and C4d positivity was found.

Discussion

It is widely recognized that CD20 positive B lymphocytes are efficient antigen presenting cells and that they provide the co-stimulation required by T cells in the traditional model of cellular rejection [13-15]. Their role in antibody-mediated rejection is not widely examined.

In this study population, the numbers of CD20 positive cells didn't correlate with age, gender or serum creatinine levels. We also didn't find statistically significant differences between CD20 cell counts in the C4d negative and C4d positive groups, which could be due to the small number of cases. This is concordant with a previous report that included only six patients with CD20 positive infiltrates and found no relationship between C4d staining and CD20 positive cell counts [16].

We found a weak correlation between the number of CD20 positive cells and the grade/severity of rejection. This could be supported by Hippen *et al* who proposed a hypothesis that local B cells could provide co stimulatory signals favoring persistence of cellular graft rejection [16]. Analyzing individual histological Banff scoring criteria, CD20 cell counts significantly correlated with the

Figure-2: Correlation between CD20 cell count and intimal arteritis (v score) (r = 0.39, p < 0.05)

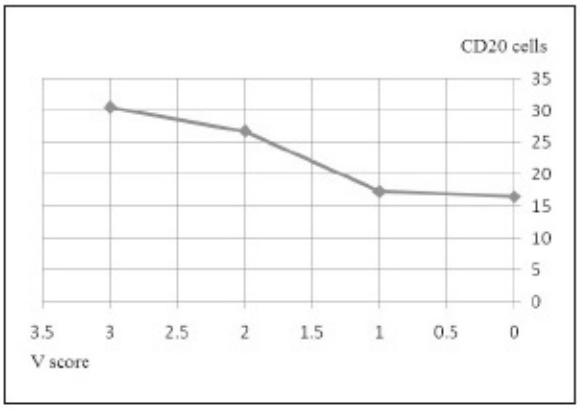
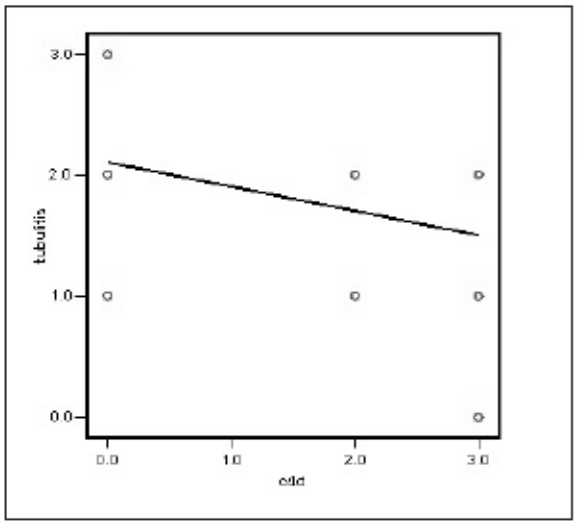


Figure-3: Correlation between C4d positivity and tubulitis score (r = -0.39, p = 0.04)



severity of intimal arteritis, reflecting that their presence within the interstitial infiltration signals a more severe form of rejection.

The presence of CD8 positive cells in this cohort study didn't correlate with any of the histological criteria of acute rejection or with the C4d or CD20 infiltrates.

Our cell counts differ from previous reports [5, 10-12] perhaps due to lack of uniform standardized criteria to define CD20 positive infiltrates. This may make it more difficult to discern the possible relationship between the presence of CD20 positive cells and the severity of rejection.

Conclusion

It seems that the presence of CD20-positive lymphocytes in the allograft interstitium among infiltrating cells in a rejection episode could represent a previously unrecognized, more severe subset of acute rejection. The question of whether they are involved in humoral rejection mechanisms is still not clearly answered and the observations obtained from this pilot study define avenues for further investigation.

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