CD20+ B-Cell Infiltration Is Related to the Time After Transplant and Poor Prognosis of Acute Cellular Rejection in Renal Transplant

Wen Jiqiu, Chen Jinsong, Cheng Dongrui, Zhang MingChao, Ji Shuming, Liu Zhi-Hong

Abstract

Objectives: This study sought to determine the relation between CD20+ B-cell infiltration and time after transplant and outcome of acute cellular rejection in renal allografts.

Materials and Methods: Fifty-five patients with acute cellular rejection were categorized into 3 groups: very early, early, and late rejection. The density of CD4+, CD8+, CD20+, and CD68+ cells and HLA-DR expression were characterized and quantified using immunohistochemical staining. Histologic changes were compared between high-density and low-density CD20+ B-cell groups. Poor prognosis factors were analyzed with Cox proportional regression.

Results: Density of CD20+ cells in the very-early rejection group was lower than it was in the early- and late-acute rejection groups (P = .03); the density of CD4+, CD8+, and CD68+ cells and HLA-DR expression did not differ between the groups. Mesangial matrix increase, tubular atrophy, arteriolar hyaline thickening, and tubulitis were more prevalent in the high CD20+ density group. Cox regression analysis demonstrated that HLA-DR expression on the tubules, arteriolar hyaline thickening, and CD20+ cell density were associated with an elevated risk of acute cellular rejection.

Conclusions: Expansion aggregation of CD20+ B cells occurred mostly after 2 weeks. When combined with HLA-DR expression and arteriolar hyaline thickening, these influence the outcome of acute cellular rejection in renal allograft.

Key words: Renal transplant, Acute cellular rejection, CD20, Graft survival, Time course

Introduction

The relation between infiltration of CD20+ B cells into the renal allograft and the outcome of acute cellular rejection (ACR) was first reported by Sarwal and associates.1 In this study, transcriptome expression patterns of biopsy samples taken from children with acute renal allograft rejection demonstrated that CD20+ was highly expressed in the steroid-resistant acute rejection group, which have a relatively poorer outcome. Immunohistochemical staining also demonstrated a correlation between dense CD20+ B-cell infiltrates and glucocorticoid-resistant rejection.1 This conclusion was later supported by other studies.2-4 A more recent report also suggests that infiltration of CD38+ B cells alone or in combination with CD38-CD20+ B cells is a predictor of poor clinical outcomes for ACR of renal allografts.5 Further, some studies have reported that anti-CD20 antibody therapy is an effective treatment for acute rejection of renal allografts associated with nodular B-cell aggregates and can improve the outcome of these types of refractory rejections.6,7 In contrast, several other reports have demonstrated that CD20+ infiltrates in renal allograft biopsies with ACR are not associated with worse graft survival.8-10 Therefore, the relation between CD20+ cell infiltration and outcomes of renal allograft is controversial.

Although the main function of B cells is the secretion of antibodies into the blood and other body...
fluids to defend against foreign invaders, they also can function as antigen-presenting cells by processing and displaying foreign peptides in a manner that can be recognized by T cells. It is unknown which of these 2 functions, if not both, are associated with CD20+ cells infiltrating the kidney during ACR.

We have noted that CD20+ B-cell–associated acute rejection lasts longer than CD20- acute rejection (unpublished data). Zarkhin and associates also reported that intragraft CD20+ cells were predominantly found when ACR occurred later after transplant (50 ± 35 months for CD20+ acute rejection vs 22 ± 23 months for CD20- acute rejection; P = .03). Notably, the outcome of late ACR is often poorer than ACR occurring early after transplant; this may be due to different mechanisms and histologic changes. Collectively, these observations prompted us to determine whether CD20+ cell aggregation correlates with the length of time between transplant and ACR.

Mononuclear cell infiltration is a common phenomenon in ACR of renal allografts. The type of mononuclear cell infiltrate, especially CD68+ cells, is also related to the outcome of ACR. This study sought to characterize mononuclear cells, especially the extent of CD20+ cell infiltration, associated with ACR. We also addressed the question of whether the degree of CD20+ cell infiltration was related to the outcome of ACR occurring at different times after transplant.

Materials and Methods

Patients
This retrospective study analyzed histologic findings and clinical data in renal allograft recipients at the Research Institute of Nephrology at Jinling Hospital. This study was approved by the Jinling Hospital Institutional Review Board for human use. Clinical characteristics and follow-up data for all patients experiencing acute rejection were assembled from the Research Institute of Nephrology Renal Transplant Registry. All protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration. Written, informed consent was obtained from all patients.

Study participants received treatment for ACR after it was confirmed by a renal allograft biopsy according to the Banff 97 classification of allograft histopathology. Type class 1A, 1B, and 2A were selected for this study. A total of 55 patients were included in this study and were separated into 3 groups according to the time between transplant and ACR: very early (< 2 weeks), early (> 2 weeks < 6 months), or late (> 6 months). Patients were further grouped based on the density of CD20+ cells detected in the biopsy. All patients were followed for more than 4 years.

All patients were subjected to antibody induction with daclizumab. Maintenance immunosuppression in these recipients included a calcineurin inhibitor (cyclosporine microemulsion or tacrolimus) in combination with mycophenolate mofetil (or mizoribine) and low-dose prednisone (5-10 mg/d). During this time, acute rejection was treated with 3 daily 500 mg boluses of intravenous methylprednisolone, followed by a 5- to 7-day oral steroid taper. Patients whose renal function did not improve within 3 to 4 days of initiation of this therapy were deemed steroid-resistant, and received additional treatment with immunoadsorption.

Biopsies
Biopsies were obtained percutaneously under local anesthesia using real-time ultrasound guidance with an 18-gauge needle core device. Biopsy specimens were fixed in formalin, stained with hematoxylin and eosin, and scored according to Banff criteria.

Immune phenotyping
The surface phenotype of infiltrating cells was determined using immunoperoxidase staining and monoclonal antibodies specific for CD20 and CD68 (Dako, Copenhagen, Denmark) to distinguish B cells from monocyte/macrophages. As a mononuclear cell, predominant acute rejection has been described to induce intense tubular class 2 expression, likely as a result of locally induced cytokines, biopsies also were stained for HLA-DR (Dako). Expression of CD8 (Dako) and CD4 (Novocastra, Newcastle upon Tyne, UK) also was assessed. A single pathologist performed the blinded assessment of immunohistochemical data using a previously established quantitative immunostaining scoring method. The immunostaining scoring method for CD20, CD68, CD4, and CD8 was performed as follows: 16 high-power field were selected, the amount of each type of mononuclear cell was calculated, and all populations were added together for the density of total mononuclear cells (per mm²). Tubular HLA-DR
staining also was evaluated and the percentage of HLA-DR+ tubules was calculated. 13

Statistics
Data are expressed as means ± SD. Differences among groups were determined using a t test, a 1-way analysis of variance, and a Student-Neuman-Keuls least-squares difference method was used for multiple comparisons. Qualitative data were described as percentages and analyzed using a chi-square or Fisher exact test where indicated. According to the distribution of the data, survival analyses were performed using the product-limit method (Kaplan-Meier), and differences between survival curves were analyzed using the log-rank test. All P values are 2-sided and a value less than .05 was considered statistically significant. Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 13.0, IBM Corporation, Armonk, New York, USA).

Results
Clinical parameters and histologic changes among the 3 patient groups are summarized in Table 1. Mononuclear cell infiltration was compared between the 3 subgroups: very early, early, and late rejection groups. The density of CD4+, CD8+, and CD68+ cells and HLA-DR expression did not differ between the 3 subgroups; however, the density of CD20+ cells was lower in the very early group compared with either the early or late groups (Figure 1).

To characterize the dynamics of T- and B-cell infiltration of the renal allograft, we further analyzed the ratio of (CD4+ + CD8+) to CD20+ cells among the 3 patient groups. The results showed that the T-cell to B-cell ratio in the very early group was higher than it was in the early and late groups (Table 2); this finding further confirms that an imbalance of T/B cell occurs at different stages of rejection.

Distribution of CD20+ cells during acute renal allograft rejection
The aggregation of CD20+ cells was mostly accompanied by CD4+ cell aggregation and HLA-DR

<table>
<thead>
<tr>
<th>Abbreviations:</th>
<th>AR, acute rejection; PRA, panel reactive antibodies</th>
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<tr>
<td>Table 1. Clinical and Histologic Parameters of the 3 Patient Groups</td>
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<tr>
<td></td>
<td>Very Early Group (Group 1, n=17)</td>
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<tr>
<td>Sex (male)</td>
<td>15</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>17</td>
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<tr>
<td>Donor type (living/deceased-donor)</td>
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</tr>
<tr>
<td>PRA at transplant</td>
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</tr>
<tr>
<td>Median age at transplant (range)</td>
<td>19-57</td>
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<tr>
<td>Median cold ischemia time (range)</td>
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<tr>
<td>Banff grade</td>
<td></td>
</tr>
<tr>
<td>1-A</td>
<td>6</td>
</tr>
<tr>
<td>1-B</td>
<td>6</td>
</tr>
<tr>
<td>2-A</td>
<td>5</td>
</tr>
<tr>
<td>Median days to AR (range)</td>
<td>4-13 d</td>
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<tr>
<td>Steroid-resistant rejection</td>
<td>3/17</td>
</tr>
<tr>
<td>Graft loss</td>
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*Graft survival was compared between receipts < 2 weeks and receipts > 2 weeks
overexpression on the tubules (Figure 2), suggesting that the CD20+ cells play a role as antigen presentation.

**Histologic changes between different CD20-density groups**
All study subjects were additionally separated into a low CD20+ cell density group and high CD20+ density group using 168 CD20+ cells/mm² tissue as the “expected” density. The rate of steroid-resistant rejection in the higher CD20+ density group was poorer than the low density of CD20+ group (12/28 vs 4/27; \( P = .048 \)), and correspondingly, the graft survival of the high CD20+ density group was poorer than the low CD20+ density group (Figure 3; \( P = .0356 \)).

Histologic changes were evaluated between high and low CD20+ density groups. An increase in mesangial matrix, tubular atrophy, arteriolar hyaline thickening, and tubulitis were more prevalent in the high CD20+ density group; CD4+, CD8+, and CD68+ and HLA-DR expression did not differ between high CD20+ density and low CD20+ density (Table 3).

**Multivariate analysis for graft survival**
Because many factors differed between the high and low CD20+ density groups, multiple Cox proportional regression analyses were performed to identify factors associated with a poor prognosis for ACR. The results demonstrate that CD20+ cell density and HLA-DR expression on the tubules, and arteriolar hyaline thickening were associated with high risks for a poor prognosis of ACR (Table 4).

**Discussion**
Our findings demonstrate an association between CD20-positive lymphocytic infiltrates of renal allografts and ACR with a poorer clinical outcome and reduced graft survival. These findings are consistent with the initial observations made in pediatric renal transplant recipients by Sarwal and colleagues. However, these findings are controversial and not in agreement with previous reports. This variance could be attributed to the use of different criteria for CD20-positive lymphocytic infiltrates, different follow-ups, and differences in study population. In our previous study (unpublished...
In the current report, we calculated the mononuclear cell infiltrate density using a quantitative method. We randomly selected 16 high-power fields (equal to 1 square millimeter) and calculated the number of mononuclear cells. This quantitative method was similar to the method used in a previous study by Sarwal and colleagues. We separated the study subjects into a high-density CD20+ group and low-density CD20+ group according to the intermediate-term of density of CD20+. Further, our quantitative method was concordant with the degree of Banff criteria; therefore, we also found that our quantitative method was in accord with the method described by Girlanda and associates in *American Journal of Transplant*.13

Graft survival in the presence of a high CD20+ cell density is not different from the low-density group when compared over the first 2 weeks of rejection. However, graft survival was different between all recipients with acute cellular rejection, because patients in the very early group were included in the low-density group. Survival of the graft in the very early group was better than the late group, which may explain the variation in the results from previous reports. CD20 cell infiltration in late rejection was not associated with C4d-positive rejection. A recent study by Hallon and associates, however, reports that C4d was a specific, but not sensitive, marker of antibody-mediated rejection. Whether the CD20+ cell infiltration during late acute rejection was related to antibody-mediated rejection should be further explored.

Conclusions

The density of CD20+ cell infiltration during very early acute rejection was low and discordant with the density of other mononuclear cells. CD20+ cell aggregation occurred mostly 2 weeks after transplant during acute rejection; the mechanism must be investigated further. CD20+ cell density, combined with HLA-DR expression and arteriolar hyaline thickening, influences the outcome of allograft survival.

References


