Abstract

Objectives: We sought to discover which types of injuries were related to human leukocyte antigen DR expression in acute rejection and late chronic injury in renal allografts.

Materials and Methods: Ninety-two recipients were separated into the early acute rejection group, the late monocyte infiltration group, and the late chronic injury group. Ten subjects with acute cellular rejection received a repeat biopsy. All samples were stained with CD4, CD8, CD20, CD68, and human leukocyte antigen DR by immunochemical staining. Levels of these markers were compared among the subgroups of each group.

Results: Human leukocyte antigen DR expression was greater in the early C4d-negative acute rejection group than it was in the early C4d-positive acute rejection group. Human leukocyte antigen DR expression was greater during acute rejection than that was on a repeat biopsy. Human leukocyte antigen DR expression was accord with the infiltration of monocyte infiltration in the acute cellular rejection group. Human leukocyte antigen DR expression was greater during late acute rejection than it was in BK virus nephropathy, which was not in accord with monocyte infiltration. Human leukocyte antigen DR expression was greater during chronic rejection than it was in IgAN, BK virus nephropathy, and TA/IF groups, and even in tubular atrophy.

Conclusions: Human leukocyte antigen DR expression in renal tubular cells was associated with early acute cellular rejection and was in accord with monocyte infiltration. Human leukocyte antigen DR expression in renal tubular cells during the late phase (especially in tubular atrophy) was a marker of chronic rejection, but was not in accord with monocyte infiltration in renal allografts.

Key words: HLA-DR expression, Acute cellular rejection, Acute humoral rejection, BK virus nephropathy, IgA nephropathy

Introduction

The main function of the human leukocyte antigen (HLA) system is to present antigenic peptides to the T-cell receptors regulating the immune response. Human class-II genes are encoded in the HLA-D region, which comprises 3 families: DQ, DP, and DR. The products of class II genes form a heterodimeric transmembrane protein consisting of a heavy α-chain and a light β-chain. The DRα chain is expressed from 1 nonpolymorphic gene, and the DRβ chain originates from 9 highly polymorphic genes. Human leukocyte antigen-DR molecules are expressed on: dendritic cells, B cells, monocytes, macrophages, precursors of myeloid and erythroid cells, and some epithelial cells. Human leukocyte antigen-D is expressed on activated T cells.

Several chronic inflammatory diseases are associated with genes in the major histocompatibility complex (MHC) class II region. For many of these diseases, this is the main genetic association. Recent genetic and functional studies support the long-held assumption that common MHC class II alleles are responsible for these disease associations. Human leukocyte antigen-DR expression on renal tubular cells has been associated with allograft rejection.
leukocyte antigen-DR expression can stimulate allogeneic lymphocytic reactions and enhance T-cell-mediated lysis. Girlanda and associates recently have shown that HLA-DR expression on tubular cells is associated with monocyte infiltration during allograft dysfunction. Identifying cellular infiltrates in that study was limited to CD68; whether it is related to CD20, CD4, or CD8 is not known.

The role of interstitial inflammation in BK nephritis is not completely understood. Interpretation of some study findings on BK nephritis have been controversial. Thus, the BK virus could hypothetically trigger rejection episodes by inducing HLA-DR upregulation, as previously proposed for the cytomegalovirus. Virally stimulated HLA-DR expression would then render this marker irrelevant for diagnosing rejection, but the latest data do not support this hypothesis. Some authors have noted that expression of HLA-DR and CD54 in urinary tubules increased in patients with decoy cell shedding, but this did not indicate concomitant acute rejection. These markers may, instead, indicate renal inflammatory activity associated with viral reactivation, which can progress to polyomavirus interstitial nephritis.

Chronic injury in renal allografts is the main cause of allograft failure. Tubular atrophy and intestinal fibrosis are the main characteristics of chronic injury. Immunologic and nonimmunologic injuries can lead to the same histologic changes in the renal allograft. Human leukocyte antigen-DR overexpression in the different phases of acute rejection is unknown.

This study focused on the relation between HLA-DR expression in renal tubular cells and the type of rejection. We also observed HLA-DR expression in chronic renal allograft injury (especially tubular atrophy), and we attempted to find a marker to distinguish immunologic injury from nonimmunologic injury.

Materials and Methods

Patients and materials

This retrospective study analyzed histologic findings and clinical data in renal allograft recipients at the Research Institute of Nephrology at Jinling Hospital (Nanjing, PR China). The study protocol was approved by the Ethics Review Board of Jinling Hospital. Clinical characteristics and follow-up data for subjects experiencing acute rejection were obtained from the Renal Transplant Registry of the Research Institute of Nephrology. All protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration, and written, informed consent was obtained from all patients.

The selected recipients underwent renal allograft biopsy according to the Banff 07 Classification of Allograft Histopathology. Ninety-two recipients with 102 biopsy samples were selected from November 2007 until March 2010. They were separated into 4 groups and then divided into 11 subgroups according to injury characteristics (see below). The demographic and clinical characteristics of the selected patients are listed in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Demographic and Clinical Characteristics of Selected Patients</th>
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<tr>
<td><strong>Number</strong></td>
</tr>
<tr>
<td>Cause of end-stage renal disease</td>
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<tr>
<td>Chronic glomerulonephritis</td>
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<tr>
<td>Diabetes mellitus</td>
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<tr>
<td>Hypertensive nephropathy</td>
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<tr>
<td>Polycystic kidney disease</td>
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<tr>
<td>IgA nephropathy</td>
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<tr>
<td>Toxicity caused by consuming Chinese herbal remedies</td>
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<tr>
<td>Other</td>
</tr>
<tr>
<td>Males</td>
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<tr>
<td>Age (y)</td>
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<tr>
<td>Time after transplant (d)</td>
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<tr>
<td>Days from transplant to biopsy</td>
</tr>
<tr>
<td>Hemodialysis/continuous ambulatory peritoneal dialysis</td>
</tr>
<tr>
<td>Han ethnicity</td>
</tr>
<tr>
<td>Living/deceased donor</td>
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<td>Serum creatinine (umol/L)</td>
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</table>

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

Biopsy

Biopsies were obtained percutaneously using local anesthesia with real-time ultrasound guidance using an 18-G needle. Biopsy specimens were fixed in formalin, stained with hematoxylin & eosin, and
scored according to the Banff 07 criteria. The surface phenotype of infiltrating cells was determined using immunohistochemical staining using monoclonal antibodies specific for CD4, CD8, CD20, and CD68 (Dako, Copenhagen, Denmark) to distinguish T cells and B cells from monocyte/macrophages. Monocyte-predominant acute rejection has been shown to induce intense tubular MHC class-II expression (probably as a result of locally induced cytokines), so biopsies also were stained for HLA-DR (Dako). Accessory molecule phenotypes for CD8 (Dako) and CD4 (Novocastra, Newcastle upon Tyne, United Kingdom) also were assessed. A single pathologist prospectively assessed cellular infiltrates by analyzing immunohistochemical samples using a previously established quantitative immunostaining scoring method. The pathologist did not know the clinical data; he calculated the number of each type of monocyte and added the density of different monocytes (per mm²). Tubular HLA-DR staining was evaluated by visually assessing the approximate proportion of tubules and calculating the percentage. The recipients with acute rejection were Banff grades I and II.

**Subgroups**

Subjects were separated into 4 groups according to Banff 07 criteria, based on the characteristics of histologic changes.

Group 1 was the early acute rejection group. Early acute rejection was defined as occurring if the acute rejection happened within 2 weeks after surgery. Group 1 was divided into 2 subgroups: early C4d-negative acute rejection (group 1A, n=13) and early C4d-positive acute rejection group (group 1B, n=7).

Group 2 was the late monocyte infiltration group. Monocyte aggregation was defined as occurring in the renal allograft if it happened more than 6 months after surgery. Group 2 was divided into 3 subgroups: group 2A (BK virus nephropathy group, n=6), group 2B (late C4d-negative acute rejection, n=12), and group 2C (late C4d-positive acute rejection group, n=13).

Group 3 was the late chronic tubular injury group. Chronic tubular and intestinal injury in the renal allograft was defined as occurring if it happened 6 months after surgery. Group 3 included 4 subgroups: group 3A (IgA nephropathy group, n=12), group 3B (BK virus nephropathy group, n=6), group 3C (chronic rejection group, n=12), and group 3D (TA/IF group, n=7).

Group 4 was the acute cellular rejection with the repeat biopsy group. Group 4 was divided into 2 subgroups: group 4A (acute cellular rejection subgroup; n=10) and group 4B (protocol subgroup; n=10).

**Analyses**

The density of CD4, CD8, CD20, and CD68 cells were compared in the subgroups of each group. Human leukocyte antigen-DR expression in renal tubules also was compared in the subgroups in each group.

In groups 1 and 2, the correlation between HLA-DR expression and monocyte infiltration also was analyzed among each subgroup in each group. In group 3, HLA-DR expression in atrophic tubules was specifically assessed to find a marker for the atrophy tubule. In group 4, the special character was that the compared samples was the same donor and the same recipients in a different period, which can exclude the influence of HLA genotype and the number of mismatches of HLA on HLA-DR expression.

**Statistical analyses**

Data are the means ± SD. Differences among groups were analyzed by the t test or a 1-way analysis of variance. The Student-Newman-Keuls or the least squares difference method was used for multiple comparisons. Qualitative data are described as percentages and analyzed using the chi-square test or the Fisher exact test. All P values are 2 sided, and P < .05 was considered statistically significant. Statistical analyses were performed using SPSS software (SPSS: An IBM Company, version 13.0, IBM Corporation, Armonk, NY, USA).

**Results**

**Group 1 (early acute rejection)**

The differential expression of HLA-DR and distribution of CD4/CD8/CD20/CD68 in early acute rejection as well as the density of CD4/CD8/CD20/CD68 density between the 2 subgroups was not significantly different. However, HLA-DR expression was greater in the C4d-negative group than it was in the C4d-positive group (P = .008; Figures 1A and B).

**Group 2 (late monocyte infiltration group)**

Human leukocyte antigen-DR expression and CD4/CD8/CD20/CD68 distribution in each
subgroup is shown in Figure 2A. There was no difference in CD4/CD8/CD20/CD68 density among the 3 subgroups. However, HLA-DR expression was lower in subgroup 2A than it was in subgroups 2B and 2C ($P = .0002$; Figures 2A and 2B).

**Group 3 (late chronic tubular injury)**

CD4/CD8/CD20/CD68 density was higher in subgroups 3B and 3D than it was in subgroups 3A and 3C ($P = .021/.047/.007/.000$), but HLA-DR expression was higher in subgroup 3D than it was in subgroups 3A, 3B, and 3C ($P = .001$; Figures 3A and 3B).

**Group 4 (acute cellular rejection with repeat biopsy)**

In group 4, ten recipients with acute cellular rejection underwent repeat biopsy, the time interval elapsed between the 2 biopsies ranged from 4 to 6 months. CD4/CD8/CD20/CD68 density and HLA-DR expression were compared with respect to acute rejection time (group 4A) and biopsy time (group 4B). CD4/CD8/CD20/CD68 density and HLA-DR expression were higher in group 4A than they were in group 4B ($P = .0036/.0018/.046/.020/.012$; Figures 4A and Figure 4B).
Correlation between HLA-DR expression and CD4/CD8/CD20/CD68 density in acute rejection

Acute rejection was in 2 parts, and represented by the acute C4d-negative rejection group (n=35) and the acute C4d-positive rejection group (n=20). In the acute C4d-negative rejection group, the density of CD8 ($P = .035$) and CD68 ($P = .004$) was correlated with HLA-DR expression. However, the density of CD4 and CD20 was not correlated with HLA-DR expression (Table 2).

In the acute C4d-positive rejection group, CD8 density also was correlated with HLA-DR expression.
(P < .001), and the density of CD4 (P = .0019) and CD20 (P = .0024) also were correlated with HLA-DR expression. However, CD68 density was not correlated with HLA-DR expression (Table 3).

Table 2. Pearson Correlation of HLA-DR Expression With CD4, CD8, CD20, and CD68 Expression in C4d-Negative Acute Rejection

<table>
<thead>
<tr>
<th>Pearson Correlation</th>
<th>Coefficients</th>
<th>P Value</th>
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<tr>
<td>CD4</td>
<td>0.1373</td>
<td>.4460</td>
</tr>
<tr>
<td>CD8</td>
<td>0.3683</td>
<td>.0350</td>
</tr>
<tr>
<td>CD20</td>
<td>0.1108</td>
<td>.5392</td>
</tr>
<tr>
<td>CD68</td>
<td>0.4826</td>
<td>.0044</td>
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</tbody>
</table>

Table 3. Pearson Correlation of HLA-DR Expression With CD4, CD8, CD20, and CD68 Expression in C4d-Positive Acute Rejection

<table>
<thead>
<tr>
<th>Pearson Correlation</th>
<th>Coefficients</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>CD4</td>
<td>0.6388</td>
<td>.0024</td>
</tr>
<tr>
<td>CD8</td>
<td>0.8970</td>
<td>.0000</td>
</tr>
<tr>
<td>CD20</td>
<td>0.6513</td>
<td>.0019</td>
</tr>
<tr>
<td>CD68</td>
<td>0.3411</td>
<td>.1410</td>
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Discussion

One study showed that expression of HLA antigens is up-regulated in acute myocarditis and may be helpful in distinguishing inflammatory myocardial diseases from noninflammatory diseases in patients with congestive heart failure. Diagnosing via HLA expression has been suggested as being superior to histologic diagnostic procedures because HLA antigens are expressed throughout the myocardium; whereas infiltrations of inflammatory cells are frequently distributed focally, and therefore may be missed during histopathologic investigations.

In this study, HLA-DR expression in the tubules of renal allografts was related to acute cellular rejection. Up-regulation of the MHC class II family (HLA-DR) and intercellular adhesion molecule 1 (ICAM-1) in tubular epithelial cells is a typical finding in graft biopsies with cellular rejection, and can serve as an adjunct diagnostic tool. Human leukocyte antigen-DR expression can stimulate an allogeneic lymphocytic reaction and enhance T-cell-mediated lysis. However, the correlation between HLA-DR expression at different times is not known, nor is the effect of HLA-DR expression on tubular atrophy. We tried to ascertain HLA-DR expression in acute and chronic injury in subjects who had undergone renal transplant.

Acute rejection at different times has different mechanisms and outcomes. C4d-negative rejection is related to cellular rejection. This study showed that HLA-DR expression was higher in cellular rejection than it was in humoral rejection. Nevertheless, late rejection did not show the same
results as those in early rejection. Human leukocyte antigen-DR expression was high in late C4d-positive rejection and C4d-negative rejection. This could be because late rejection was related to T-cell–mediated activation, which stimulated HLA-DR expression on tubular epithelial cells. It has been reported that HLA-DR expression is related to interstitial monocyte infiltration. Also, BK virus nephropathy has been characterized by interstitial infiltration of inflammatory cells, but HLA-DR expression in BK virus nephropathy is low, results which are in accord with our data. Therefore, HLA-DR expression was not in complete accord with interstitial infiltration of inflammatory cells. Human leukocyte antigen-DR expression appeared only in the immunologic injury seen in renal allografts.

Chronic injury to tubules is seen in late renal allograft loss; tubular atrophy is seen in > 80% of allografts 6 months after transplant. Distinguishing late injury in the allograft is difficult. A marker of tubular atrophy can be used to distinguish different types of injury. Therefore, we selected 4 types of late chronic rejection tubular to ascertain different HLA-DR expressions. Human leukocyte antigen-DR expression was higher in the chronic rejection groups than in the IgA nephropathy, AT/IF, and BK virus nephropathy groups. This result suggests that HLA-DR overexpression could be used as an immunologic marker for late chronic injury to renal tubules. Such an immunologic marker could be helpful in distinguishing immunologic and nonimmunologic injuries in the late renal allograft loss.

In this study, 10 individuals with acute cellular rejection were selected. They were treated effectively and also received repeat biopsy before or after acute rejection. The biopsy period and the acute rejection period can be considered as 2 times. Human leukocyte antigen-DR expression during the acute rejection time was obviously higher than it was during the biopsy period; a similar tendency was seen with respect to CD4/CD8/CD20/CD68 infiltration. Therefore, we consider that HLA-DR overexpression appeared only during the phase of rejection, and that HLA-DR expression decreases once effective therapy for the rejection is administered. Human leukocyte antigen-DR overexpression also can be used as a marker for acute rejection.

Human leukocyte antigen-DR expression was high in the late acute C4d-positive subgroup, and HLA-DR expression was high in chronic rejection and late acute rejection. However, HLA-DR expression was low in the IgA nephropathy, the AT/IF, and the BK virus nephropathy groups. Human leukocyte antigen-DR expression could be used as a marker of immunologic injury during the late period, and also can be used to distinguish nonimmunologic injury in late renal allografts.

The present study has shortcomings. The category of each group and subgroup was in accordance with Banff 07 criteria, but the diagnosis of each group could be controversial (eg, AT/IF group). We tried to select the typical pathological changes and hoped that these would reflect the true nature of renal allografts. We used a quantitative method to describe the density of cell infiltration and HLA-DR expression. We compared the results of our method to those of published data: good accord was observed. The pathologist was the same in all groups, thus eliminating interobserver variations.

The Banff classification system for acute rejection is used to assess the degree of cellular infiltration without differentiating the phenotype of the infiltrate. Furthermore, the combined contribution of different cell types has been investigated: these studies have correlated increasing heterogeneity with worsening outcome. More recently, experimental and clinical studies have reported on the role of macrophages during acute rejection. In particular, Grimm and associates have shown that activated macrophages and their products characterize acute dysfunction during rejection compared with normal histology and subclinical rejection. Kajiwara and associates have reported a ratio of common antigens to CD68/leukocyte, increased expression of HLA DR, and increased levels of granulocyte-monocyte colony stimulating factor during acute rejection compared with borderline changes and chronic rejection.

The present study showed that the density of CD8 and CD68 was correlated with HLA-DR expression. However, the density of CD4 and CD20 was not correlated with HLA-DR expression in the acute C4d-negative rejection group. However, in the acute C4d-positive rejection group, the density of CD8 was correlated with HLA-DR expression, and the density of CD4 and CD20 was correlated with HLA-DR expression. However, the CD68 density was not correlated with HLA-DR expression, a result which was in accord with the study of Girlanda and associates. The mechanism of this association should be investigated further.
In conclusion, HLA-DR expression in renal tubular cells was associated with early acute cellular rejection and in accordance with monocyte infiltration in renal allografts. Human leukocyte antigen-DR expression in renal tubular cells during the late period (especially with respect to tubular atrophy) was a marker of late rejection, and was not in accordance with monocyte infiltration in renal allografts.

References