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Donor-specific antibodies present at the time of kidney transplantation in immunologically unmodified patients increase the risk of acute rejection

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ABSTRACT

Background: Human leukocyte antigens (HLA) class II donor-specific antibodies (DSAs) are associated with microcirculation inflammation, transplant glomerulopathy and ultimately graft loss. There is however no data on allograft outcomes in deceased donor kidney transplant recipients who have *not* received any desensitization prior to transplantation.
Methods: We prospectively evaluated the association of HLA DR and DQ DSAs on rejection and short-term graft survival in patients who did not receive desensitization prior to transplantation. On the basis of their cumulative strength of HLA DR and/or DQ DSA, the patients were dichotomized into: 1) median fluorescence intensity (MFI) < 1000 and 2) MFI ≥ 1000.
Results: In the two year study period, 50 consecutive patients with HLA DR and/or DQ sensitization were transplanted in our two centers. Post-transplantation, the incidence of acute rejection was significantly greater in the MFI ≥ 1000 group (35%; 8/22) compared to the MFI < 1000 group (7%; 2/28) ($p < 0.001$). There were two graft losses, both in the MFI ≥ 1000 group.
Conclusion: The strength of DR and/or DQ DSA at the time of renal transplantation influences the risk of rejection in non-desensitized recipients with HLA class II DSA.

1. Introduction

Abundant evidence suggests that HLA class II donor-specific antibodies (DSAs) that are present in a renal transplant recipient before transplantation are detrimental for long-term renal allograft outcome [1–4]. They are associated with microcirculation inflammation, transplant glomerulopathy and ultimately graft loss [2,3,5]. In one study [2], the incidence of transplant glomerulopathy (TG) was 5% in patients without class II DSA, 18% in the group of patients with lower levels of class II antibody (MFI < 2000), 38% in those with stronger levels of class II antibody (MFI 2000–10,000) and 36% in patients with MFI's over 10,000 [1]. Higher class II antibody levels has been associated with C4d-positivity in peritubular capillaries (PTC), and which in turn has been associated with TG and worse graft survival. Finally, *de novo* DQ DSA's are associated with a particularly high risk for antibody mediated rejection (AMR) and ultimately TG [4].

After the emergence of the association of transplant glomerulopathy with class II DSAs in the literature [1–3], we began to evaluate the role of class II DSA's have on renal graft outcome. In the past, transplant centers, including the ones in this study, often proceeded with the transplant despite a positive B cell crossmatch [6,7]. Screening for HLA class II antibodies by single-antigen bead (SAB) testing was not routinely performed to help inform whether to accept the organ offer or desensitize the recipient before transplantation. However, after implementing SAB testing in 2007 we started evaluating the outcome of deceased donor

Abbreviations: AMR, antibody mediated rejection; DSA, donor specific antibodies; HLA, human leukocyte antigen; MFI, median fluorescence intensity; PRA, panel reactive antibody; PTC, peritubular capillaries; SAB, single-antigen bead; TG, transplant glomerulopathy.

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renal transplant candidates who were HLA class II sensitized at the time of transplant. We evaluated the impact of the strength of class II DSA's on graft function on outcome in 50 consecutive HLA class II sensitized patients who received a deceased donor kidney in our two transplant centers from 2010 to 2012. This study differs from others evaluating the role of class II DSAs on graft outcome as none of our patients were desensitized prior to transplantation. This study specifically allows for the evaluation of the effect of class II DSA levels on graft outcome independent of desensitization therapy or class I DSA.

2. Methods

2.1. Study description/patient selection

In this study, we evaluated the effect of the pre transplant class II DSA strength on early graft rejection and short-term graft outcome. During the study period, all patients undergoing deceased donor transplantation from the two participating transplant centers were routinely screened for HLA class I and II antibodies by a mix and/or panel reactive antibody (PRA) Luminex assay (One Lambda, Inc., Canoga Park, CA). If class II antibody activity was detected by these screening assays, a class II SAB assay was performed after the patient was transplanted. The SAB results were not available to the transplant centers prior to the transplant. These results were solely used for the analysis of outcomes associated with the presence or absence of class II DSA. Informed consent was not obtained from the patients since at that time, neither center selected patients for transplants based on the B cell crossmatch or presence of class II DSA.

All consecutive class II sensitized patients with HLA class II antibody detected by SAB and who received a deceased donor transplant from May 2010 to August 2012 were included in the study. The patients were arbitrarily divided into 1) median fluorescence intensity (MFI) < 1000 and 2) MFI ≥ 1000 groups based on cumulative levels of DR/DQ DSA measured after the transplant from the serum obtained prior to the transplant. A MFI of 1000 was used as an arbitrary cut off as DSA < 1000 is often considered acceptable for transplant offers [8]. The serum sample chosen for class II DSA/SAB testing was the sample obtained closest to, but before the transplantation. We also assessed if the first 20 consecutive patients with class II antibodies were capable of fixing the C1q component of complement using the One Lambda C1q assay. Only 20 patients underwent this testing as we had limited kits available. Since we did not find any correlation with MFI or rejection, we did not extend this testing to other patients.

2.2. Cumulative HLA class II DSA strength

Each patient's HLA class II DSA strength was defined as the cumulative sum of each of the patient's class II DSA's present in the serum sample tested as previously demonstrated by Wiebe et al. [9]. For example, if a patient had the DSA's DRB1*04 and DRB4*01 with MFI levels of 2000 and 5000, respectively, the cumulative class II DSA level would be 7000 MFI. We used the cumulative sum of DSA's and not the immune-dominant DSA, since we assumed that each of a patient's class II DSA's could bind equivalently to class II donor antigens in the kidney. In cases where there were more than one SAB per DR or DQ antigen, we selected the bead with the highest MFI.

2.3. Immunologic testing criteria used for proceeding with transplantation

The immunologic decision of whether or not to transplant each patient was based on the patient having a negative T cell flow cytometry crossmatch. Patients were not transplanted if they had a positive T cell flow cytometry crossmatch. A positive B cell crossmatch was not used to determine the decision to transplant. Complement dependent cytotoxic crossmatch was not done. If a patient had any class I antibodies (A, B and C) as detected by class I SAB prior to the transplant, they were entered into UNet as unacceptables; but class II antibodies were not. Each patient's calculated class II PRA (cPRA) was determined for HLA DR and DQ, but not DP, antibody activity, using 2989 local deceased donor class II (DR and DQ) phenotypes our laboratory had molecularly-typed from 2007 to 2011. The class I cPRA was similarly calculated from our local donors. Finally, most of the donors and candidates were typed at the low resolution level (serological equivalent level) using molecular methods (SSP/SSOP) for A, B, C, DRB1, 3, 4 and 5 and DQB1. HLA DP DSA could not be evaluated because most of the deceased donors were not DP typed.

2.4. Immunosuppression

All patients received standard induction with anti-thymocyte globulin or basiliximab along with mycophenolate mofetil and intravenous steroids. Calcinuerin inhibitors were started 2–3 days after transplantation. Initiation of calcinuerin inhibitors was delayed in recipients with delayed graft function. These patients were continued on anti-thymocyte globulin until kidney function improved. Patients with high PRA (irrespective of the DSA and/or a positive B cell crossmatch) also received high dose intravenous immunoglobulin at 2 g/kg in divided doses at the time of transplantation, followed by 1 g/kg of intravenous immunoglobulin at 1, 3 and 12 months after transplantation. Maintenance immunosuppression consisted of mycophenolate mofetil 1 g/day and tacrolimus (dose targeted to maintain a trough level of 8–10 ng/dl in the first three months and 6–8 ng/dl thereafter) or cysclosporine (dose targeted to maintain a two hour peak level of 800–1000 ng/dl in the first three months and 600–800 ng/dl thereafter).

2.5. Antibody-mediated rejection (AMR) and cellular rejection; diagnosis and treatment

All acute rejections were biopsy-proven with histological classification assigned according to the Banff '07 criteria. Biopsies were performed for clinical indications such as increase in serum creatinine, delayed graft function, or proteinuria. Cellular rejection was treated with anti-thymocyte globulin and intravenous steroids for 5–7 days. AMR was treated with high dose intravenous immunoglobulin (2 g/kg in divided doses) and intravenous steroids. Intravenous immunoglobulin at 1 g/kg was repeated at 1, 3 and 12 months after initial treatment. In some refractory patients, plasmapheresis, bortezomib (1.3 mg/m^2 on days 1, 4, 8 and 11) and/or rituximab (375 mg/m^2) were also used to treat AMR. Calcineurin inhibitors were continued during the treatment of either type of rejection.

2.6. Statistical analysis

Statistical analysis employed contingency table analysis, logistic regression and various descriptive proportions. Two-sided paired t tests were used to compare continuous variables and chi-square test was used for dichotomous variables. A p-value of < 0.05 was considered statistically significant. Cumulative incidence of rejection was analyzed in the two MFI groups. Odds ratio for risk of rejection with MFI ≥ 1000 was calculated using binary logistic regression.

3. Results

3.1. Patient groups

Of the patients transplanted from May 2010 to August 2012 in the two participating transplant centers, there were 50 consecutive patients who showed evidence of sensitization to HLA class II antigens before transplantation, as evidenced by a positive class II antibody screening assay (mix or PRA). The average number of days before transplant that the serum sample was collected and used to test for class II DSA was 16 days. There were 28 patients in the MFI < 1000 group with a

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