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Histopathologic changes in anti-angiotensin II type 1 receptor antibody-positive kidney transplant recipients with acute rejection and no donor specific HLA antibodies

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ABSTRACT

Objective: To determine the association of antibodies against angiotensin II type 1 receptor (AT1R Ab) and histopathologic changes seen in patients with kidney allograft rejection and negative donor specific HLA antibodies (DSA).
Methods: Stored sera from 27 patients who had biopsy-proven rejection in the absence of DSA were tested for AT1R Ab. Biopsy slides of all patients were re-examined and classified according to Banff 2013 criteria. Histopathologic changes were compared between AT1R positive and negative patients.
Results: 75% of patients with positive pre-transplant AT1R Ab had antibody mediated rejection (AMR) compared to 37% of AT1R Ab-negative patients. A trend towards increased interstitial inflammation was observed in the AT1R Ab positive group ($p = 0.08$). More patients in the AT1R Ab positive group had microcirculation inflammation (88% vs 58% with glomerulitis scores ≥ 1; 75% vs 58% with peritubular capillaritis scores ≥ 1).
Conclusion: In kidney transplant recipients with rejection and no DSA, a higher incidence of AMR and worse inflammation scores are observed in the presence of positive pre-transplant AT1R antibodies.

1. Introduction

Since the seminal paper by Patel and Terasaki in 1969 on the significance of a positive crossmatch test in kidney transplantation [1], improved HLA typing and antibody testing have contributed to a better understanding of alloimmunity. Preformed donor specific HLA antibodies (DSA) have been associated with increased risk of antibody mediated rejection (AMR) [2]. At the University of Pennsylvania, our practice of careful pre-transplant HLA antibody analysis of potential recipients and avoidance of transplantation across positive crossmatches, coupled with the use of induction therapy predominantly with rabbit anti-thymocyte globulin (rATG), have resulted in a low incidence of allograft rejection (<10%), despite transplantation of high-immunologic risk recipients, including highly sensitized or African American recipients. About a third of these rejections occurred in the absence of detectable anti-HLA DSA.

Antibodies to AT1R (AT1R Abs) have been associated with AMR and worse graft outcomes in patients without anti-HLA DSA [3–11]. However, the exact pathophysiology leading to poor graft outcomes in patients with AT1R Abs is unclear and there is limited data on the histopathologic changes seen in patients with and without AT1R Abs. Using the recommended cut-off set by the manufacturer of the ELISA (≥ 17 U/ml as positive), we sought to describe histopathologic correlates of AT1R Ab detected prior to transplantation and at time of rejection. We describe the clinical implications of these correlates and the potential effect of therapeutic options.

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2. Materials and methods

2.1. Study design

This is a retrospective cohort analysis of kidney transplant recipients with biopsy-proven rejection and no anti-HLA DSA. Stored pre-transplant sera and sera obtained around the time of kidney biopsy were tested retrospectively for AT1R antibody. The study was approved by the Institutional Review Board at the University of Pennsylvania.

2.2. Subjects

Between January 1, 2010 and December 31, 2014, four hundred and forty-six kidney transplant recipients followed at the University of Pennsylvania had biopsies for one or more of the following reasons: creatinine rise, hematuria or proteinuria. Of the 102 patients who were listed to have biopsy-proven rejection, anyone who had the following were excluded: positive *de novo* anti-HLA DSA (n = 62), non-donor directed HLA antibodies that were cross-reactive with DSA (n = 1), no stored sera (n = 8) and no biopsy slides available for review (n = 1). On review of biopsy slides, three patients who had changes that were not attributable to rejection (1 had FSGS, 2 had interstitial fibrosis) were also excluded. The remaining 27 patients who had stored pre-transplant and post-transplant sera and had rejection confirmed on re-examination of biopsy slides were included in this final analysis.

2.3. HLA antibody detection and flow cytometric crossmatch

Pre-transplant immunologic testing for all patients were performed in the HLA laboratory at the Hospital of the University of Pennsylvania as previously described [12] and included recipient and donor HLA typing, recipient anti-HLA DSA determination and flow cytometric crossmatch (FCXM) between donors and recipients. HLA typing of recipients and donors was performed using DNA based techniques and included HLA-A, -B, -C, -DRB1, -DRB3/4/5, and -DQB1. In addition, if the recipient had antibodies against HLA-DQA, or -DPB, typing of donor and recipient for these loci was also performed. Anti-HLA antibody testing was performed using multiple Luminex platforms including phenotype beads and single antigen bead assays (One Lambda, Canoga Park, CA). Assays were performed according to the instructions provided by the manufacturer except for the addition of dithiothreitol to sera for the purpose of reducing interference. Reactivity due to denatured epitopes and non-specific reactivity detected by the single antigen bead assays were ruled out by a careful analysis of the specificity pattern of bead reactivity as well as constancy of reactivity among the different Luminex bead assays. DSA specificities included HLA-A, B, C, DRB1, B3, B4, and B5, DQA/DQB, and DP. A positive anti-HLA DSA was defined as a normalized MFI value >1000 and any increase above pre-transplant MFI levels. Any patient with anti-HLA DSA from historic or current blood samples were disqualified from receiving a transplant from the corresponding donor. T and B cell FCXM were performed using a Beckman-Coulter FC500 (1024 channels). For sample preparation, cells were treated with Pronase 1 mg/ml and stained with affinity purified F(ab′)_2 Goat anti-IgG, Fcγ, 2.2 mol FITC per mole F(ab′)2 (1:160 dilution). In most cases, serum specimens used in the final crossmatch included a current serum drawn within the last 30 days prior to transplantation. FCXM reactivity was expressed using a relative ratio of Molecules of Equivalent Soluble Fluorescence values of the tested sample over the negative control sample. FCXM was deemed negative if the fluorescence ratio is <1.5, weakly reactive if the fluorescence ratio is 1.5–2.5 and positive if the fluorescence ratio is >2.5. Prior to reporting, the FCXM results are reviewed for consistency with past and current anti-HLA antibody specificities, presence of autoantibodies or other potentially interfering substances. Only pairs with a negative crossmatch were allowed to undergo transplantation. Post-transplant, all patients were further tested for anti-HLA antibody within 30 days of a kidney biopsy.

2.4. AT1R antibodies

AT1R antibody detection was performed by ELISA (One Lambda, Canoga Park, CA) in the Immunogenetics Laboratory at Johns Hopkins University. Pre and post-transplant sera were obtained before any treatment for rejection was initiated. Sera were diluted at 1:100 prior to testing and added in duplicate wells onto the microtiter plate coated with human AT1R extracted from transfected Chinese hamster ovary cells. Following a two-hour incubation, plates were washed and incubated with a secondary antibody goat anti-human IgG labeled with horseradish peroxidase for detection. A standard curve was used to determine antibody concentration. A cut off of 17 U/ml was used to distinguish AT1R Ab positive from AT1R Ab negative.

2.5. Histopathologic evaluation of kidney biopsy

All slides were re-examined by a single renal pathologist for consistency and interpreted based on Banff 2013 criteria [13]. The pathologist was blinded to the results of AT1R Ab analysis. Acute rejection was scored based on: tubulitis (t), intimal arteritis (v), glomerulitis (g), peritubular capillaritis (ptc), interstitial inflammation (i), total interstitial inflammation (ti) and C4d staining. Borderline rejection was reported for histologic indices t1/i1-2 or i1/t1-2. Any t or i score more than borderline was classified as T-cell mediated rejection (TCMR). A diagnosis of histologic AMR was made if both histologic evidence of acute tissue injury (microvascular inflammation with g > 0 and/or ptc > 0 or intimal or transmural arteritis with v > 0 or acute thrombotic microangiopathy, in the absence of any other cause of acute tubular injury) and evidence of current/recent antibody interaction with vascular endothelium (linear C4d staining in peritubular capillaries, at least moderate microvascular inflammation ([g + ptc] ≥2) were both present. The amount of chronic allograft injury was scored based on: interstitial fibrosis (ci), and tubular atrophy (ct), chronic glomerulopathy (cg), vascular intimal thickening (cv) and arteriolar hyaline thickening (ah).

2.6. Kidney transplant immunosuppression protocol

Induction therapy generally consisted of three to five doses of rabbit anti-thymocyte globulin (rATG, Thymoglobulin; Genzyme, Cambridge, MA) at 1.5 mg/kg/dose, titrated to recipient immunological risk. Recipients with very low immunologic risk received two doses of basiliximab at 20 mg/dose (Simulect; Novartis Pharmaceuticals Corp., East Hanover, NJ). Maintenance immunosuppression comprised tacrolimus, mycophenolic acid (MPA) agent and prednisone. Target tacrolimus trough levels were 8–12 μg/L for the first 12 weeks post-transplant, 6–8 μg/L for the next 12 weeks, 5–7 μg/L until 1 year post-transplant, then 4–6 μg/L thereafter. Rarely, cyclosporine-modified (CsA) was used for patients intolerant of tacrolimus. Patients received methylprednisolone 500 mg intravenous bolus intraoperatively, then oral prednisone on post-operative day 1, tapered down to 5 mg daily by 30 days post-transplant.

2.7. Statistical analysis

All statistical analyses were performed using Stata/MP version 13.1 (Statacorp., College Station, TX). Descriptive statistics (median