AJKD
Original Investigation

Isolated De Novo Antiendothelial Cell Antibodies and Kidney Transplant Rejection

Elena Sánchez-Zapardiel, MSc,[1] Esther Mancebo, PhD,[1,2] María Díaz-Ordoñez, BSc,[3] Lucía de Jorge-Huerta, BSc,[3] Lara Ruiz-Martínez, BSc,[3] Antonio Serrano, MD, PhD,[1,2,4] María J. Castro-Panete, PhD,[1,2] Alberto Utrero-Rico, BSc,[2,3] Amado de Andrés, MD, PhD,[5] José M. Morales, MD, PhD,[2,5] Sara Domínguez-Rodríguez, BSc,[3] and Estela Paz-Artal, MD, PhD[1,2,3,4]

Background: Studies analyzing the role of antiendothelial cell antibodies (AECAs) in large series of kidney transplant recipients are scarce, and HLA, MHC (major histocompatibility complex) class I−related chain A (MICA), and angiotensin II type 1 receptor have not been formally excluded as targets.

Study Design: Retrospective study of a cohort of kidney transplant recipients.

Setting & Participants: 324 kidney transplant recipients who were negative for anti-HLA, anti-MICA, and anti–angiotensin II type 1 receptor antibodies were tested for AECAs in pre- and posttransplantation serum samples.

Predictors: AECA-positive (preformed [pre$^+$/post$^+$] vs de novo [pre$^-$/post$^+$]) versus AECA-negative (pre$^-$/post$^-$) before or after transplantation.

Outcomes: Patient mortality, transplant loss, and acute rejection events.

Results: 66 (20%) patients were AECA positive (39 [12%] preformed, 27 [8%] de novo) and 258 (80%) were AECA negative. During a follow-up of 10 years, 7 (18%) AECA pre$^+$/post$^+$ patients had rejections compared with 14 (52%) AECA pre$^-$/post$^+$ and 57 (22%) AECA pre$^-$/post$^-$ recipients (OR, 3.80; $P = 0.001$). AECA pre$^-$/post$^+$ status emerged as an independent risk factor for transplant rejection compared to the AECA pre$^-$/post$^-$ group (OR, 5.17; $P < 0.001$). However, AECA pre$^+$/post$^+$ and AECA pre$^-$/post$^+$ patients did not show higher risk for either patient death (ORs of 1.49 [$P = 0.7$] and 1.06 [$P = 0.9$], respectively) or transplant loss (ORs of 1.22 and 0.86, respectively; P for both = 0.8) compared to the AECA pre$^-$/post$^-$ population.

Limitations: Retrospective study. Posttransplantation sera were collected before or after rejection, entailing a nearly cross-sectional relationship between the exposure and outcome. Lack of identification of precise antigens for AECAs.

Conclusions: De novo AECAs may be associated with rejection. These antibodies might serve as biomarkers of endothelium damage in kidney transplant recipients.

Am J Kidney Dis. 68(6):933-943.

INDEX WORDS: Anti-endothelial cell antibodies (AECAs); de novo antibodies; allograft rejection; immunofluorescence patterns; kidney transplantation; acute rejection; allograft loss; mortality.

Kidney transplant recipients can develop allo- and/or autoantibodies, before and after transplantation, that may contribute to transplant deterioration and eventual loss. HLA, MHC (major histocompatibility complex) class I−related chain A (MICA), and angiotensin II type 1 receptor (AT_1R) are well-recognized targets for antibody responses in patients who underwent transplantation. Due to their polymorphic nature, HLA and MICA alloantigens frequently elicit humoral responses, anti-HLA and anti-MICA antibodies being major contributors for antibody-mediated rejection.[1] Anti-AT_1R antibodies (probably both allo- and autoreactive) may cause rejections, characterized by prominent vascular features.[2,3]

Antiendothelial cell antibodies (AECAs) are a heterogeneous family of antibodies that recognize different antigens on endothelial cells. Although AECAs have been mainly related to the development of systemic autoimmune diseases,[4] they have also been detected in heart and kidney transplant recipients. Regarding kidney transplantation, several case reports describe the presence of these antibodies in the serum of patients having hyperacute

From the [1]Department of Immunology and [2]Research Institute I+12, Hospital Universitario 12 de Octubre, Madrid, Spain; [3]School of Medicine, Universidad Complutense de Madrid, Madrid, Spain; [4]Section of Immunology, Universidad San Pablo CEU, Madrid, Spain; and [5]Department of Nephrology, Hospital Universitario 12 de Octubre, Madrid, Spain.

Received January 19, 2016. Accepted in revised form July 6, 2016. Originally published online September 3, 2016.

Address correspondence to Elena Sánchez-Zapardiel, MSc, Avda de Córdoba s/n, 28041, Madrid, Spain. E-mail: eszapardiel@gmail.com

0272-6386
http://dx.doi.org/10.1053/j.ajkd.2016.07.019

rejection.[5-8] Nevertheless, AECAs have been mainly associated with acute rejection and early transplant loss.[9-15] Acute rejection episodes are more frequently described as multiple[12,13] or severe[14] among AECA-positive recipients. In the study of a cohort of 226 kidney transplant recipients, Sun et al[15] found that de novo AECAs were associated with higher risk for steroid-resistant rejection episodes. They also demonstrated an association of de novo AECAs with the presence of glomerulitis and peritubular capillary inflammation and no correlation with C4d deposition at the time of kidney biopsy. Furthermore, AECAs have been related to decreased long-term transplant survival and increased frequency of chronic allograft nephropathy.[12]

Despite these reports that associate AECAs with deterioration of the kidney transplant, whether AECAs cause rejection or arise after previous damage of the transplant is not known. Based on the presence of de novo AECAs before rejection in 3 of 4 post-transplantation serum samples obtained from HLA-negative kidney transplant recipients, Ronda et al[10] reported that AECAs can directly cause rejection. Using proteomic techniques to identify the target proteins for AECAs,[16] 9 autoantigens (eg, nucleolin, vimentin, and α-tubulin) and alloantigens (eg, MICA and human keratin 1) have been found in sera of anti-HLA−negative kidney transplant recipients who showed positive reactions with a panel of human umbilical vein endothelial cells (HUVECs) by flow cytometry. In a recent analysis,[17] high-density protein arrays were used to investigate sera of endothelial cell crossmatch-positive kidney recipients undergoing antibody-mediated rejection without donor-specific anti-HLA antibodies. Four different antigenic targets expressed on vascular endothelium (endoglin, Fms-like tyrosine kinase 3 ligand, EGF-like repeats and discoidin I-like domains 3, and intercellular adhesion molecule 4) were identified.

In this report, we have analyzed the prevalence of preformed and de novo AECAs in a cohort of kidney transplant recipients without anti-HLA, anti-MICA, or anti-AT_1R antibodies. Information for AECA antigen targets has been obtained by immunofluorescence analysis in HUVECs. We demonstrate that de novo AECAs, directed against either cytoskeleton or nuclear antigens, are independently associated with transplant rejection.

METHODS

Patients and Samples

We retrospectively analyzed 727 patients (452 men and 275 women; mean age, 54 ± 16 [standard deviation] years) who received a kidney transplant between 2005 and 2011 at Hospital 12 de Octubre. The initial cohort has been previously described.[18] Characteristics of all 727 patients are summarized in Table S1 (provided as online supplementary material). A final cohort of 324 recipients was analyzed. Figure 1 shows the cohort selection and antibodies tested for each group. We retrospectively tested posttransplantation AECAs, selecting samples either close to the occurrence of a rejection event (mean time posttransplantation, 15 [range, 1-48] months) or between the first month and fourth year after transplantation among rejection-free patients (mean time posttransplantation, 20 [range, 1-49] months). Anti-HLA and anti-MICA antibodies were measured in both pre- and post-transplantation samples. Anti-AT_1R antibodies were tested in AECA-positive posttransplantation sera. Delayed graft function (DGF) refers to the requirement of dialysis in the first week posttransplantation. Serum creatinine levels ≤ 1.20 mg/dL for men and ≤0.90 mg/dL for women were considered to reflect optimal kidney function. Transplant loss and patient mortality were considered to calculate transplant and patient survival, respectively. Transplant rejection was defined as biopsy-proven

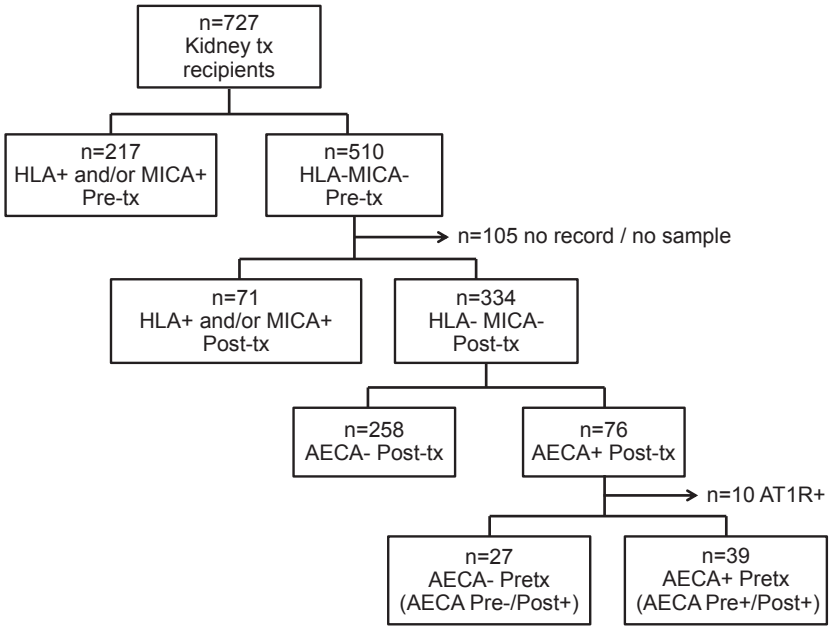

Figure 1. Flow diagram for cohort selection and antibodies measured within each group. Anti-HLA antibodies (HLA), anti–major histocompatibility complex class I−related chain A antibodies (MICA), and antiendothelial cell antibodies (AECAs) were tested in pre- (Pre-tx) and posttransplantation (Post-tx) serum samples. Angiotensin II type 1 receptor (AT_1R) antibodies were tested in AECA-positive samples to discard their potential effect on transplant evolution.