Human Immunology (2007) 68, 652–660

journal homepage: www.elsevier.com/locate/humimm

Kidney graft recipients with pretransplantation HLA CLASS I antibodies and high soluble CD30 are at high risk for graft loss

Libia M. Rodríguez[a], Sara C. París[a], Mario Arbeláez[b,c], José M. Cotes[d], Caner Süsal[e], Yolanda Torres[d], Luís F. García[a,*]

[a] *Grupo de Inmunología Celular e Inmunogenética, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia*
[b] *Departmento de Medicina Interna, Sección de Nefrología, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia*
[c] *Unidad Renal, Hospital Universitario San Vicente de Paúl, Medellín, Colombia*
[d] *Instituto de Ciencias de la Salud, Medellín, Colombia*
[e] *Department of Transplantation Immunology, University of Heidelberg, Heidelberg, Germany*

Received 13 December 2006; received in revised form 14 April 2007; accepted 11 May 2007

KEYWORDS
Kidney transplantation;
HLA antibodies;
Soluble CD30;
Graft survival

Summary In the present study, we investigated whether pretransplantation HLA class I and class II antibodies and pretransplantation levels of soluble CD30 (sCD30) and IgA anti-Fab autoantibodies are predictive of kidney allograft survival. Pretransplantation sera of 504 deceased-donor kidney recipients were tested for IgG HLA class I and class II antibodies, sCD30, and IgA anti-Fab levels using the CTS 4 ELISA kit. Kidney graft survival was estimated by Kaplan-Meier method and multivariate Cox regression. Regardless of the presence of HLA class II antibodies, recipients with high HLA class I reactivity had lower 1-year graft survival than recipients with low reactivity ($p < 0.01$). Recipients with high sCD30 had lower 5-year graft survival rate than those with low sCD30 ($p < 0.01$). The sCD30 effect was observed in presensitized and nonsensitized recipients, demonstrated a synergistic effect with HLA class I antibodies ($p < 0.001$), and appeared to be neutralized in recipients with no HLA class II mismatches. IgA anti-Fab did not influence kidney graft survival. Our results indicate that high pretransplantation sCD30 levels and HLA class I positivity increase the risk of kidney graft loss regardless of other factors. Consequently, such determinations should be routinely performed to estimate recipients' risks of graft rejection before transplantation.

Introduction

Allograft survival depends on a number of immunologic factors that determine whether the graft will be rejected. These factors include HLA matching between donor and recipient, the pretransplantation sensitization status of the recipient, and the ability of the recipient to recognize and respond to donor antigens. The presence of donor-specific HLA antibodies is a well-recognized factor of poor prognosis for graft survival; however, not all HLA antibodies represent a similar risk for graft loss. It is well known that HLA class I antibodies are a high risk factor for rejection, whereas the clinicalrelevance of HLA class II antibodies is still controversial [1–4].

* Corresponding author. Fax: + 57 4 210 6455.
E-mail address: lfgarcia@udea.edu.co (L.F. García).

0198-8859/$ -see front matter
doi:10.1016/j.humimm.2007.05.004

ABBREVIATIONS

CTS	Collaborative Transplant Study
DTT	dithiothreitol
ELISA	enzyme-linked immunoabsorbent assay
HLA	human leukocyte antigen
IgA	anti-Fab immunoglobulin A anti-Fab
IgG	immunoglobulin G
O.D.	optical density
sCD30	soluble CD30

Besides donor-specific antibodies, the presence of panel reactive antibodies in pretransplantation serum also represents a risk factor for graft survival [1,2,5,6]. Collaborative Transplant Study (CTS) data show that the presensitization against both HLA class I and class II of first kidney transplant recipients results in increased rejection of HLA mismatched grafts, whereas the pretransplantation presence of HLA class II antibodies in absence of HLA class I antibodies did not influence graft survival [7].

Another immunologic parameter associated with better graft survival is serum level of Immunoglobulin A (IgA) anti-Fab [8–10]. Even in the presence of HLA antibodies patients with high pretransplantation levels of these autoantibodies have a better survival rate than those with low levels. In addition, this effect is strengthened according to the degree of HLA matching [10–12].

Recently, evidence has been gathered regarding the T-cell activation marker soluble CD30 (sCD30) as an independent predictor of immunologic risk in renal transplant recipients without preformed alloantibodies [13–17]. This evidence suggests that the measurement of sCD30 levels offers relevant clinical information regarding rejection risk and can contribute to the selection of the appropriate immunosuppresive regimen in high-risk recipients for the prevention of acute rejection and chronic allograft nephropathy [13,14,16].

The aim of this study was to determine the predictive power of pretransplantation HLA class I and II antibodies, sCD30, and IgA anti-Fab status in deceased-donor kidney transplant outcomes in patients undergoing transplantation at a single transplantation center in Medellin, Colombia. Results show that in donor-specific crossmatch negative recipients, high levels of pretransplantation sCD30 and non-donor-specific anti-HLA antibodies are important risk factors for poor kidney transplant outcome.

Subjects and methods

Patients and donors

The study included 504 recipients of kidneys from deceased donors transplanted at the Hospital Universitario San Vicente of Medellín, Colombia, between January 1999 and December 2001, whose immediate pretransplantation sera were stored in our laboratory. All recipients had a negative pretransplantation T- and B-cell crossmatch with their specific donor, using dithiothreitol (DTT)-treated sera. Recipient selection was done based primarily on HLA-A, -B, and -DR matching. Of the patients, 308 (61.1%) were male. Among patients of both genders, the median age at transplantation was 40.5 years (interquartile range, 30–51 years). The cause for renal failure was unknown for the majority of patients (32.5%). Among known causes, the most frequent were nonspecific glomerulonephritis (16.1%), diabetes (13.7%), and congenital renal malformations (11.5%). The most frequent type of renal replacement therapy was hemodialysis (302 patients, 59.9%). A total of 101 patients (20%) underwent both hemodialysis as well as peritoneal dialysis before the transplantation, 53 (10.5%) underwent peritoneal dialysis only, and 48 (9.5%) did not receive any replacement therapy. The median pretransplantation dialysis time was 6 months (range, 1–18 months).

The total number of deceased organ donors was 316, of which 252 (80%) were male and 64 (20%) female. The median age at the time of death was 26 years (interquartile range, 20–36 years). The most frequent blood type was O (62.7%) donors, followed by A (29.7%), B (7%), and AB (0.6%). The median cold ischemia time was 23 hours (interquartile range, 17–28 hours).

Of the patients, 481 (95.4%) underwent transplantation for the first time; 22 (4.4%), for the second time, and one for the third time. The median for pretransplantation blood transfusion was two (interquartile range 0–4 transfusions), and 429 (85.1%) had three or more HLA mismatches with their respective donors. The immunosuppressive therapy received by these patients included steroids in 504 (100%) individuals, azathioprine in 490 (97.2%), cyclosporine in 490 (97.2%), mycophenolate mofetil in 24 (4.8%), and rapamune in 6 (1.2%). Only 33 (6.5%) patients received induction therapy, of whom 21 were treated with OKT3, 11 with Basiliximab, and only one patient was treated with antithymocyte globulin (ATG)/antilymphocyte globulin (ALG).

Throughout the observation time between December 1999 and June 30, 2005, there were 44 (8.7%) patients lost to follow-up, six (1.2%) graft failures because of surgical complications, 18 (3.6%) graft failures for medical reasons other than rejection (including infection and recurrence of the original disease), 64 (12.7%) graft losses because of acute or chronic graft rejection (defined by clinical criteria, alterations in renal function, and histologic findings in kidney biopsy), and 71 (14.1%) deaths for reasons unrelated to the transplants.

Donor-specific crossmatch

Donor-specific crossmatch was done by the classical complement-dependent cytotoxicity (CDC) using recipient dithiothreitol (DTT)-treated fresh serum [18]. Briefly, donor spleen mononuclear cells were isolated by Ficoll-Hypaque (Sigma Chemical, St. Louis, MO), and separated into T and B lymphocytes using nylon columns (Robbins Scientific, Sunnyvale, CA). T and B cells (3×10^3 per well) were loaded onto Terasaki plates (Robbins, Scientific) with 1 μl/well of recipient DTT-treated serum during 30 minutes for T lymphocytes and 60 minutes for B cells, at 4°, 20°, and 37°C. Thereafter, 5 μl/well of rabbit complement (supplemented with 0.002 mol/L L-cysteine) were added, and the trays with T and B cells were incubated for 60 minutes and 90 minutes, respectively. Stain Fix (eosin) was used to stain dead cells. Scores $\leq$2 ($\leq$20%) were considered negative.

Determination of HLA class I and II antibodies, sCD30, and IgA anti-Fab serum levels

Pretransplantation serum levels of IgG HLA class I and II antibodies, sCD30, and IgA anti-Fab autoantibodies were determined by enzyme-linked immunoabsorbent assay (ELISA) using the CTS 4-ELISA Kit (University of Heidelberg, Heidelberg, Germany) according to the manufacturer's instructions. HLA class II antibodies could not be determined in three patients because of insufficient serum. Briefly, serum was diluted 1:4 for sCD30, 1:2 for IgG