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Heightened expression of HLA-DQB1 and HLA-DQB2 in pre-implantation biopsies predicts poor late kidney graft function

Karina L. Mine[a], Hélio Tedesco-Silva[b], Tuíla B. Mourão[a], Erika F. Campos[a], Larissa A. Salzedas[a], Bruna Aguiar[a], Claudia R. Felipe[b], Jose O. Medina-Pestana[b,c], Maria Gerbase-DeLima[a,c,*]

[a] Instituto de Imunogenética – AFIP, Rua Loefgreen 1235, 04040-031 São Paulo, SP, Brazil
[b] Hospital do Rim e Hipertensão, Rua Borges Lagoa 960, 04038-002 São Paulo, SP, Brazil
[c] Universidade Federal de São Paulo, Rua Sena Madureira 1500, 04021-001 São Paulo, SP, Brazil

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ABSTRACT

Background: Accurate pre-transplant prediction of late graft function remains an unmet need in kidney transplantation. The aim of this study was to evaluate HLA genes expression levels in pre-implantation biopsies (PIB) of deceased donor kidneys as markers for long-term graft outcome. *Methods:* HLA genes expression analysis was initially performed using microarray data of 53 PIB, previously generated by our laboratory. The validation analysis was performed by real-time PCR in 116 PIB from an independent cohort. *Results:* The microarray data showed association between high expression levels of HLA class II genes, especially HLA-DQB1 and -DQB2, in kidneys from young (18 to 49-year-old) donors and poor (eGFR < 45 mL/min/1.73 m^2) 1- and 5-year graft function. A subsequent study in an independent cohort, in which only HLA-DQB2 expression was evaluated, validated the association between increased HLA-DQB2 expression in PIB of kidneys from young donors and poor 1-year graft function: expression levels ≥ 0.0025 relative units conferred an odds ratio of 22.5, with positive and negative predictive values of 71.4% and 90.0%, respectively. *Conclusion:* Heightened expression of HLA-DQB1 and -DQB2 in PIB are promising tools for pre-transplant risk assessment of poor late graft function in transplants with kidneys from 18 to 49-year-old donors.

1. Introduction

Despite significant advances in our understanding of the mechanisms of allograft rejection and improvements in immunosuppressive regimens that led to a steadily increase in early and late kidney graft survival rates, improving long-term graft survival remains one of the critical challenges facing kidney transplantation [1].

Assessment of the risk for graft loss prior to transplantation could be important both for kidney allocation and for individualization of post-transplantation management, which could result in better graft survival.

Considering that the immune response to human leukocyte antigens (HLA) is the key factor that ultimately leads to allograft rejection [2–8], it is of interest to investigate whether heightened HLA expression in the graft, a factor that enhances the immunogenicity, correlates with rejection and poor graft evolution.

Immunohistochemical studies in renal allograft biopsies have demonstrated that rejection episodes are accompanied by HLA-DR antigen expression on structures that do not constitutively express them and by a markedly increase expression on structures that normally express HLA-DR antigens in low levels, especially tubular epithelial cells [9–13]. A study that also examined HLA-DQ and -DP antigens expression showed that, in contrast to HLA-DR expression, HLA-DQ and -DP expression was weak or absent and without association with rejection [14].

Two studies analyzed the relationship between HLA-DR antigen expression, by immunohistochemistry in biopsies collected before the transplant, and graft outcomes. Koo et al. [15] evaluated biopsies before implantation and observed association between increased expression of HLA-DR, either isolated or in combination with elevated levels of ICAM-1 and VCAM-1, and early acute rejection, but not with graft function at three and six months post-transplant. Nyhof et al. [16] analyzed biopsies performed at the time of explantation (zero-time biopsies) and observed that the degree of HLA-DR expression significantly correlated with graft loss during the first year after transplantation. Unfortunately, none of these results were validated in

Abbreviations: Ct, cycle threshold; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; nEU, normalized expression units; NPV, negative predictive value; PCR, polymerase chain reaction; PIB, pre-implantation biopsy; PPV, positive predictive value; *TBP*, gene encoding TATA-box-binding protein

* Corresponding author at: Rua Loefgreen 1235, 04040-031 São Paulo, SP, Brazil.
E-mail address: gerbase@igen.org.br (M. Gerbase-DeLima).

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independent cohorts.

The present study aimed to investigate, for the first time, the role of HLA genes expression levels in pre-implantation biopsies (PIB) as markers for mid-term and late graft dysfunction. To this end, we initially analyzed HLA genes expression in a large scale gene expression dataset previously generated by our laboratory (GenBank accession no. GSE54888) and, subsequently, we sought to validate the preliminary results in a different cohort of transplants using real-time polymerase chain reaction (real-time PCR) for HLA gene expression determination. Furthermore, we tested the utility of using HLA expression levels for predicting graft dysfunction.

2. Materials and methods

2.1. Patients and samples

All patients were recipients of first kidney transplants performed in our center.

For the microarray analyses we used 53 pre-implantation biopsies of kidneys from transplants performed between 2008 and 2010, which were included in a previous study [17]. In the validation analysis, we used 116 PIB from transplants performed in 2014 and 2015. All transplant recipients signed an informed consent, approved by the local ethics committee. The transplants were performed with kidneys from heart-beating deceased donors and the inclusion criterion was the availability of a pre-implantation biopsy fragment adequate for gene expression analysis. Exclusion criteria were recipient's age less than 18 years and peak or current calculated panel reactive antibodies (PRA) above 80%. All transplants were performed with a pre-transplant negative complement-dependent cytotoxicity crossmatch against donor T and B cells and without HLA-A,-B or -DR donor-specific antibodies (DSA) with mean fluorescence intensity higher than 1500 in the Luminex single antigen bead assay (One Lambda, Thermo Fisher Scientific, Canoga Park, CA). The presence of HLA-DQ DSA in current or historic sera did not contra-indicate the transplant but, when a DSA was detected, a flow cytometry crossmatch was performed immediately after the transplant and all the results were informed to the transplantation team.

The immunosuppressive therapy consisted of a calcineurin inhibitor combined with prednisone and either azathioprine, mycophenolic acid or sirolimus. Some recipients from the microarray study group received induction therapy with basiliximab or anti-thymocyte globulin. All recipients from the validation group received induction therapy with anti-thymocyte globulin due to a change in the immunosuppressive protocol at our center. The baseline characteristics of the two cohorts are presented in Tables 1 and 2.

2.2. Graft outcome

The renal function was evaluated using the estimated glomerular filtration rate (eGFR), calculated using the abbreviated MDRD formula [18]. The cases with eGFR less than 45 mL/min/1.73 m^2 comprised the low eGFR group and those with eGFR ≥ 45 mL/min/1.73 m^2 comprised the high eGFR group. This eGFR cutoff was the same used in other studies [19–21].

2.3. Statistical analysis of clinical data

Analyses were performed with GraphPad Prism® 5.0 (GraphPad Software, Inc, La Jolla, CA) and PASW Statistics version 18.0 (SPSS Inc., Chicago, IL). Categorical variables were analyzed using Fisher's exact test, whereas continuous variables were analyzed with Student's *t* test or Mann-Whitney test. Stepwise logistic regression was used to test the independence of clinical variables associated with low eGFR. For correlation between variables, we used the Person correlation test. Values of $p < 0.05$ were considered statistically significant.

Table 1
Baseline characteristics of the transplants used in HLA expression analyses on microarray data, according to the estimated glomerular filtration rate (eGFR) at 1 year post-transplantation.

Patient and donor characteristics	eGFR at 1 year post-transplant	
	Low eGFR (< 45 mL/min/1.73 m^2)	High eGFR (≥ 45 mL/min/1.73 m^2)
Number of patients	18	35
Recipient age, years, mean (SD)	45.3 (8.9)	50.4 (11.4)
Recipient gender, male, %	55.6	65.7
Donor age, years, mean (SD)	50.6 (12.9)*	38.4 (12.9)
Donor gender, male, %	55.6	60.0
Last donor creatinine, mg/dL, median (min–max)	1.3 (0.5–5.4)	1.5 (0.4–3.8)
Delayed graft function, %	50.0	48.6
Extended criteria donor[a], %	44.4*	17.1
Acute rejection, %	27.8	14.3
Cold ischemia time, hours, mean (SD)	26.0 (7.1)*	21.9 (6.0)
Induction therapy with ATG or Basiliximab, %	72.2	60.0
HLA-A,-B,-DR mismatches, mean (SD)	2.4 (1.5)	2.3 (1.6)
HLA-DR 0 mismatch, %	77.8	77.1

Abbreviations: SD: standard deviation; ATG: anti-thymocyte globulin.
[a] According to the United Network for Organ Sharing definition.
* p value < 0.05, in the univariate analyses: Fisher's exact test for categorical variables; *t* test or Mann Whitney test for continuous variables. When the three variables that were significant different were tested with logistic regression, only donor age remained independently associated with low eGFR at 1 year post-transplant (p = 0.005).

Table 2
Baseline characteristics of the transplants used in microarray and validation groups.

Patient and donor characteristics	Microarray group	Validation group (real-time PCR)
Number of patients	53	116
Recipient age, years, mean (SD)	48.7 (10.8)	48.4 (11.3)
Recipient gender, male, %	62.3	59.5
Donor age, years, mean (SD)	42.6 (14.1)	45.4 (12.6)
Donor gender, male, %	58.5	57.8
Last donor creatinine, mg/dL, median (min-max)	1.3 (0.4–5.4)	1.6 (0.3–11.8)
Delayed graft function, %	49.1	49.1
Extended criteria donor[a] %	26.4	26.7
Acute rejection, %	18.9	10.3
Cold ischemia time, hours, median (min-max)	23.5 (8.3–37.0)	23.0 (11.9–39.0)
Induction therapy with ATG or Basiliximab, %	64.2	100.0*
HLA-A, -B, -DR mismatches, mean (SD)	2.3 (1.5)	2.3 (1.1)
HLA-DR, 0 mismatch, %	77.4	85.3
HLA-DQ donor specific antibody (MFI > 300)	0/47 (0%)	4[b] (3.4%)

Abbreviations: SD: standard deviation; ATG: anti-thymocyte globulin.
Statistics: Fisher's exact test for categorical variables; *t* test or Mann Whitney test for continuous variables.
[a] According to the United Network for Organ Sharing definition.
[b] All antibodies with MFIs > 2000.
* p value < 0.05.

2.4. Sample collection and RNA isolation

Kidney graft biopsies were performed immediately before implantation using an 18-gauge needle and the fragments were immediately immersed in RNAlater® (Thermo Fisher Scientific, Waltham,