Donor Specific Antibodies Median Fluorescence Intensity Levels Are the Best Indicator for Monitoring Desensitization Treatment in Kidney Transplant

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INTRODUCTION

Donor-specific human leukocyte antigen (HLA) antibodies (DSA) are increasingly recognized as a risk factor for kidney transplant graft failure. Nowadays, new serum screening methods have greatly enhanced the detection and specificity analysis of anti-HLA class II antibodies in sensitized patients.[1,2] Panel-reactive antibodies (PRA) rate has historically been performed by complement-dependent cytotoxicity (CDC) method.[3] In this respect, many histocompatibility laboratories have changed PRA for calculated reaction frequency (CRF) and implemented Single Antigens Beads (SAB) analysis.[3] Additionally, different protocols that use B lymphocyte-depleting molecules (anti-CD20), intravenous immunoglobulin (IVIG) and plasmapheresis (PP) have also been developed.[4]

This work reports a kidney transplant case of an antibody-mediated rejection (AMR) that has been treated with a desensitization protocol (plasmapheresis/IVIG) and anti-CD20. Luminex-based antibody detection results (mean fluorescence intensity; MFI) were consistent with therapy effectiveness, whereas luminex PRA/CRF outcomes were not informative.

CASE REPORT

A 69-year-old Caucasian woman was transplanted in our hospital with a kidney from an age-matched deceased donor (71 years old). The transplant was performed with total HLA-A, -B

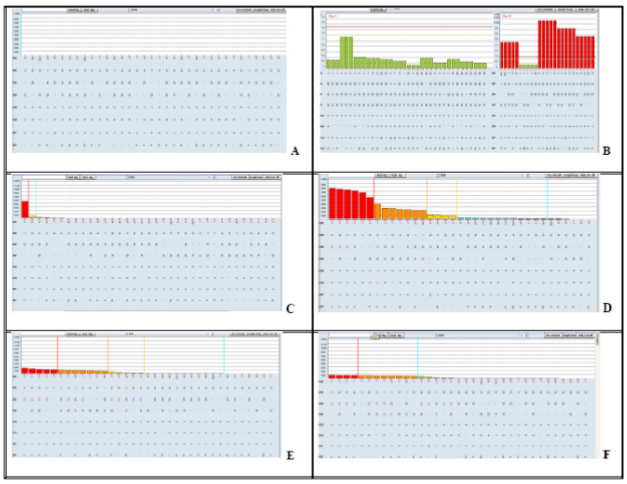

Figure . (A) Luminex pre-transplant data showing a PRA (Panel-Reactive Antibodies)/CRF (Calculated Reaction Frequency) = 0% for HLA class II screening. The patient did not present antibodies; (B) Luminex Mix post-transplant data [15th day PTP (Post-Transplantation Period)] positive for HLA class II. Anti-HLA class II antibodies did appear in this second screening; (C) Luminex PRA post-transplant data (15th day PTP) showing a PRA/CRF = 3% in specificities analysis; (D) Luminex PRA post-transplant data (22nd day PTP) showing a PRA/CRF = 37%; (E) Luminex PRA/CRF post-transplant with Rituximab treatment data (45th day PTP) showing a PRA/CRF = 34%, without appreciable variation compared with the previous determination; (F) Luminex PRA/CRF post-transplant with Rituximab treatment data (52th days PTP) showing a similar PRA/CRF = 34%, indicating reversion of humoral rejection.

and -DR incompatibility. The donor-recipient HLA typings were: A*01, *02; B*07, *18; DRB1*01, *04 for the donor, and A*11, *26; B*38, *44; DRB1*13, -, for the recipient. Microbeads array-luminex (One Lambda HD kits, Canoga Park, CA, USA) and CDC techniques showed a PRA level of 0%, which confirmed that the patient, who did not have any previous transplant or pregnancy history, was not sensitized to HLA antigens (Figure, A). Before the transplantation, CDC cross-matching (CM) was negative.

The maintenance immunosuppressive regimen consisted of prednisone (Dacortin; Merck Farma y Química, Barcelona, Spain), mycophenolate mofetil (Cellcept, F. Hoffman-La Roche, Basel, Switzerland) and tacrolimus (Prograf; Astellas, Killorglin Co., Kerry, Ireland), as previously published.[5]

On the 15th day of the post-transplantation monitoring period (15th day PTP), we detected de novo anti-HLA class II antibodies (IgG) with Luminex (One Lambda, Canoga Park, CA, USA) (Figure, B). Anti-HLA class I and anti-MICA antibodies screening by luminex was negative. A goat anti-human IgG coupled with phycoerythrin was used for antibody detection. With the luminex analyzer (LabScan), reporter fluorescence intensity of each bead was determined and expressed as mean fluorescence intensity (MFI) which is directly proportional to the amount of antibody bound to the microspheres. MFImax is defined as the highest MFI level. The cut off value was calculated using negative sera (blood group AB sera from 35 non-transfused healthy males). A mean value and 3 standard deviations were calculated with a cut-off value of 3.0 and allowing an ambiguous area from 2.5 to 3.0, as previously reported.[5]

Additionally, we detected anti-DQB1*03:02 (donor had this allele) antibodies with MFImax ≅ 5673 and PRA/CRF = 3% (LS2PRA, LabscreenPRA, OL, CA, USA) (Figure, C), and with an apparently normal level of creatinine (Cr = 1.0 mg/dL) and proteinuria (Pr = 1.2 g/24hr). One week later (22th day PTP), the patient had developed significant instability in renal function [(Cr = 2.1 mg/dL) and proteinuria (Pr = 6.5 g/24hr)], and was highly positive (MFImax 7986 ≅ and PRA/CRF = 37% [single antigen (SA) = 10%]) for anti-DRB1*04 and -DQB1*03:02 (donor typing) (LS2A01, OL, CA, USA) (Figure, D).

Moreover, we performed CM of post-transplantation B-cells and pre- or post-transplant sera, by CDC assay. The results showed that CM was negative for pre-transplant serum, and positive for post-transplant. The CM test was performed as previously published.[3,5] In addition, the sera sample were tested against a panel of B cells from frozen donor spleens (23 donors) by CDC screening, showing negative (PRA = 0%) and positive (PRA = 8.6%-17.4%) results for pre-transplant and post-transplant serum, respectively.

Based on these facts, nephrologists from our hospital indicated a renal biopsy which was positive for C4d deposition (diffuse staining), compatible with humoral allograft rejection.

The patient was pulsed with methylprednisolone (three 500 mg boluses), multiple plasmapheresis (three sessions a day, every 5 days) and IVIG post-session (0.25 g/kg; 1 gr/kg in the last session), from the 22nd day PTP on, without functional improvement. One week later, the MFImax data was not different to those obtained before the treatment.

Thus, we administered 500 mg anti-CD20 (Rituximab, Roche pharmaceuticals, Basel, Switzerland) intravenously

(two doses on the 36th and 43rd days PTP) and the initial clinical response was highly favorable. Biopsies performed on the 45th day PTP (post-anti-CD20 administration) appeared to show reversion of the humoral rejection [(Cr = 1.4 mg/dL) and proteinuria (Pr = 3.2 g/24hr)]. Analysis of the serum on the 45th day PTP revealed that the MFImax of the anti-donor DR4 had fallen to 2021 (PRA/CRF = 34%, SA = 10%) (Figure, E) and seven days later to MFImax ≅ 1176 (PRA/CRF = 34%, SA = 10%) (Figure, F). Despite the outcome of the treatment, all samples in this study showed similar PRA/CRF and SA levels (34-37% and 10%, respectively). Thus, the results suggest that the recognized antigens are always the same, regardless of the antibody concentration in serum.

To date, there has been an improvement in the patient's condition (an increased diuresis), although the renal function improves very slowly [(Cr = 1.4 mg/dL) and proteinuria (Pr = 2.7 g/24hr)]. The renal ultrasound scan shows a normal renal graft and the renal gammagraphy shows a good perfusion.

DISCUSSION

Few articles compare the different methods of HLA antibodies screening[1,2] for the detection of HLA antibodies. Indeed, current HLA class II matching strategies in kidney transplantation consider only the serologically defined HLA-DR antigens controlled by the DRB1 locus, although mismatching for HLA-DQ and HLA-DP appears associated with lower graft survivals and the development of clinically relevant alloantibodies in transplant recipients.[2,5.6]

Consequently, HLA-specific antibodies found in post-transplantation patients have been shown to be strongly associated with allograft failure.[6] Thus, de novo DSA in serum present at the time of a biopsy increases the probability of microcirculation inflammation and damage lesions, and a subsequent graft loss. Consistent with recent publications,[4,7] our data shows that the majority of de novo DSA are usually DSA II.

De novo DSA is associated with microcirculation changes typical of microcirculation inflammation (glomerulitis, peritubular capillaritis) and deterioration (transplant glomerulopathy, mesangial matrix increase, peritubular capillary basement membrane multilayering) and with diffuse C4d staining.[7] Accordingly, our patient presented C4d diffuse staining.

In heart transplant patients, a similar predominance of DSA II and an association with cardiac allograft vasculopathy and decreased graft survival can be observed,[8] as well as an increased risk of rejection and coronary artery disease.[9] Similarly, anti-class II DSA are associated with bronchiolitis obliterans syndrome in lung transplantation.[10]

With respect to our case, all samples showed a very similar luminex PRA/CRF level (34-37%), regardless of the treatment outcome. Only MFI levels showed a correlation with the treatment evolution and were predictive of the clinical outcome of the patient.

Thus, this case underlines the importance of identifying patients who develop de novo post-transplant antibodies by using very sensitive screening methods, and emphasizes the importance of MFI quantification over of PRA/CRF (still demanded by some clinicians) and SA percentages. In fact, Luminex technology is regarded as the most sensitive and safest method for antibody detection and is preferred over other antibody-detection techniques.[3,7]

CONCLUSION

MFI levels, quantified by luminex method, are a better indicator for monitoring the development of humoral rejection than PRA/CRF/SA percentages.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Muro M, Llorente S, Marín L, et al. Acute vascular rejection mediated by HLA antibodies in a cadaveric kidney recipient: discrepancies between FlowPRATM, ELISA and CDC vs luminex screening. Nephr Dyal Transplant. 2005;20:223-6.

2. Mizutani K, Terasaki PI, Hamdani E, et al. The importance of anti-HLA-specific antibody strength in monitoring kidney transplant patients. Am J Transplant. 2007;7:1027-31.

3. Bosch A, Llorente S, Diaz JA, et al. Low median fluorescence intensity could be a nonsafety concept of immunologic risk evaluation in patients with shared molecular eplets in kidney transplantation. Hum Immunol. 2012;73:522-5.

4. Gloor JM, Sethi S, Stegall MD, et al. Transplant glomerulopathy: Subclinical incidence and association with alloantibody. Am J Transplant. 2007;7:2124-32.

5. Muro M, González-Soriano MJ, Salgado G, et al. Specific "intra-allele" (IA) and "intra-broad antigen" (IBA) HLA alloantibodies in kidney graft transplantation. Hum Immunol. 2010;71:857-60.

6. Mao Q, Terasaki PI, Cai J, et al. Extremely high association between appearance of HLA antibodies and failure of kidney grafts in a five year longitudinal study. Am J Transplant. 2007;7:864-71.

7. Hidalgo LG, Campbell M, Sis B, et al. De novo donor-specific antibody at the time of kidney transplant biopsy associates with microvascular pathology and late graft failure. Am J Transplant. 2009;9:2532-41.

8. Kaczmarek I, Deutsch MA, Kauke T, et al. Donor-specific HLA alloantibodies: Long-term impact on cardiac allograft vasculopathy and mortality after heart transplant. Exp Clin Transplant. 2008;6:229-35.

9. Lietz K, John R, Burke E, et al. Immunoglobulin M- to immunoglobulin G anti-human leukocyte antigen class II antibody switching in cardiac transplant recipients is associated with an increased risk of cellular rejection and coronary artery disease. Circulation. 2005;112:2468-76.

10. Palmer SM, Davis RD, Hadjiliadis D, et al. Development of an antibody specific to major histocompatibility antigens detectable by flow cytometry after lung transplant is associated with bronchiolitis obliterans syndrome. Transplant. 2002;74:799-804.