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Brief Communication

HLA-C antibodies are associated with irreversible rejection in kidney transplantation: Shared molecular eplets characterization

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ABSTRACT

We report an interesting case concerning an irreversible antibody-mediated rejection (AMR), associated with anti-HLA-C DSA, which occurred after a second kidney transplantation despite having determined a low number of antibodies directed against HLA-C antigens (MFI < 1000) in the previous transplantation (which was then considered to be an indicator of low risk of AMR). A 63-year-old woman was re-transplanted with pre-transplant (PrT) sensitization. On day 7 post-transplantation, oligoanuria occurred and increased MFIs for the detected PrT antibodies and other antibodies (non-detected or detected with very low PrT MFI) were observed. SAB assay also showed antibodies against the second donor HLA-C mismatches and other HLA-C antigens. Nephrologists suspected AMR and the patient was therefore treated with methylprednisolone/plasmapheresis/IVIG/anti-CD20 without improvement, which led to transplantectomy. Histologic analysis confirmed acute AMR. Interestingly, it was possible to define exactly the potential immunizing epitopes whose recognition determines the specific antibody production. So, 1st donor DSAs (detected PrT with low MFI), 2nd donor DSAs (detected PTP), and non-DSA detected PTP have several shared eplets, being the 11AVR eplet the only one present on all alleles. Thus, the recognition of 11AVR eplet in the first transplant modeled the patient's antibody response. Therefore, we propose that donor HLA-C typing should always be performed for recipients with anti-HLA-C antibodies, and specific shared-eplets should be investigated in order to determine previous transplant mismatches.

1. Introduction

The donor-specific (HLA) antibody (DSA) response to a renal allograft is not fully understood [1]. In recent years, new serum-screening methods have greatly enhanced the detection and specificity analysis of anti-HLA antibodies in sensitized patients. HLA antibodies (Abs) showed to be usually epitope- and not antigen-specific (Ag-specific). Patients who lost a graft developed multiple antibody patterns that are specific for the HLA molecules that share the donor's mismatched epitopes. The clinical significance of anti-HLA-C DSAs in renal transplantation is even more unclear [2–6]. Recent studies showed acute antibody-mediated rejection (AMR) and transplant glomerulopathy in the presence of anti-HLA-C DSA antibodies [4,5]. Moreover, donor HLA-C typing is not usually performed prospectively by transplant staff members on-call in Europe.

This work reports a kidney transplant case that resulted in irreversible AMR associated with anti-HLA-C DSA. The anti-HLA-C antibodies caused rejection with shared eplet mechanisms. In the first transplant, a low number of antibodies directed against HLA-C antigens was determined by luminex median fluorescence intensity (MFI) levels (<1000), which indicated low risk of AMR; however, they produced irreversible AMR after the second transplant. These low titer anti-HLA-C antibodies that shared specific eplets with second donor could have an important role on the transplant's outcome.

Abbreviations: HLA, human leukocyte antigen; DSA, donor-specific antibody; IVIg, intravenous immunoglobulin; PRA, panel-reactive antibodies; CDC, complement dependent cytotoxicity; CM, cross-matching; PrT, pre-transplant; PTP, post-transplant period; MFI, mean fluorescence intensity.

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2. Case description

A 63-year-old woman with end-stage renal failure due to nephroangiosclerosis was re-transplanted with a kidney from a deceased donor who did not have any unacceptable antigens. The typings were: recipient-HLA-A*02:01, *11:01; B*35:01, *51:01; C*04:01, *15:01; DRB1*11:01, *13:01; DQB1*03:01, *06:03; and donor-HLA-A*02:01, *03:01; B*44:02, *57:01; C*05:01, *06:02; DRB1*07:01, *13:01; DQB1*02:02, *06:03. Microbeads array-luminex (LabScreenMix, OneLambda, CA) technique showed a PRA level of 5%, which indicated that the patient, who had had a previous transplant (2-years ago) performed with HLA-A33, -A68, -B14, -Cw8, -DR1 and -DQ5 mismatches (first donor: HLA-A*33:01, *68:02; B*14:01, *14:02; C*08:01; DRB1*01:01, *11:01; DQB1*03:01, *05:01), was pre-transplant (PrT) sensitized to HLA antigens. The organ was not nephrectomized. 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 expressed as MFI which is directly proportional to the amount of antibody bound to the microspheres. MFI higher than 1000–1500 are usually considered positive, as commonly accepted [7–10]. MFI_{max} is defined as the highest MFI level. All tests were performed as previously published [7,8].

Prior to transplantation, our patient was only luminex-positive for anti-A68 (MFI = 1549), anti-DR1 (MFI = 1935) and anti-DQ5 (MFI = 1836) against the incompatible antigens from the first transplant. Anti-A33 and anti-B14 (mismatches with the first donor) were also considered positive and unacceptable, although their MFI was <1000. Indeed, her last transfusion was performed in 2007 and she has not since been pregnant. Thus, the final pre-transplant detected sensitized antigens were only HLA-A68, -DR1 and -DQ5 with $MFI_{max} \cong 1935$.

Before the transplantation, cross-matching (CM) by standard CDC (complement dependent cytotoxicity) assay was negative.

The maintenance immunosuppressive regimen consisted of prednisone (Dacortin, Merck, Spain), mycophenolate mofetil (CellCept, Roche, Switzerland) and tacrolimus (Prograf, Astellas, Ireland), as previously published [5].

After increased diuresis without complete renal function recovery, oligoanuria occurred on day 7 of the post-transplantation period (7th day PTP). Kidney magnetic resonance imaging was normal. Graft biopsy was not performed because of disorders of homeostasis. Therefore, recipient serum was subjected to an emergency luminex SAB assay (LS1A04 and LS2A01, OL, CA).

Increased MFIs were observed for the detected PrT antibodies, such as anti-A68 (MFI = 1801) and anti-DR1 (MFI = 2063), as well as for other antibodies that had not been detected or had been detected with a very low PrT MFI (<1000) previously, such as anti-B*14:02 (MFI = 4348), anti-B*14:01 (MFI = 5721), anti-B*18:01 (MFI = 5586) and anti-C*08:01 (MFI = 3720) (Table 1). The SAB assay showed antibody reactivity against donor HLA-C mismatches including HLA-C*05:01 (MFI = 5825) and -C*06:02 (MFI = 3100), and other antigens such as HLA-C*07:01 (MFI = 2408), -C*12:02 (MFI = 2127) and -C*18:01 (MFI = 3701). Anti-C*05:01 and anti-C*06:02 were DSAs (present in the second donor).

Post-transplantation CM by CDC (with extended times for more sensitivity) and flow cytometry (FC) assays, showed negative and positive results for pre- and post-transplant sera (PrT and PTP), respectively, as previously reported [7,8]. A positive FCCM was identified when the sample MFI exceeded that of negative control values by 3 SD. We also tested to what extent prozone might have masked the presence of antibodies prior to transplant. Therefore, we tested neat and 1:8 and 1:16 titer in parallel and all results were apparently similar.

Based on these facts, nephrologists suspected AMR. The patient was pulsed with methylprednisolone (three 500 mg boluses), plasmapheresis (three sessions a day, every five days) and IVIG (0.25 g/kg and the last session 1 g/kg) without functional improvement. Ten days later, the MFI_{max} data post-PP/IVIG was not different to those obtained before the treatment (Table 1).

Thus, we administered 500 mg anti-CD20 (Rituximab, Roche pharmaceuticals) intravenously (two doses on the 20th PTP) and the initial clinical response was not favorable. Nor was the MFI_{max} data obtained on days 25th and 28th PTP different to previous data (Table 1).

On day 40th a cortical necrosis was diagnosed, leading to transplantectomy. Histological analysis showed focal necrosis of the cortical parenchyma, important vascular lesions and positive peritubular C4d staining, which confirmed grade III of acute AMR. The post-transplantectomy serum samples (50th day) showed the highest MFI values and 15 days later these MFI values clearly decreased (Table 1).

All pre- and post-transplant samples were tested twice and MFI values were reproducible (±200 MFI units). The values shown in Table 1 correspond to the first determination.

Interestingly, it was possible to define the potential immunizing epitopes eplets whose recognition determines the specific antibody production. To identify the epitope recognized by these alloantibodies, we analyzed and compared the aminoacid sequences of the patient's HLA class I molecules with the HLA alleles corresponding to antibodies observed pre- and post-transplant, by using an online database (http://www.ebi.ac.uk/imgt/hla) (HLA Informatics group at the Anthony Nolan website), Histochek, HLA-Matchmaker and RasMol v2.2 (Molecular Graphics Visualization Tool, Edinburgh) (ftp://hpux.csc.liv.ac.uk/hpux/X11/Viewers/RasMol-2.5.1), as previously published [7,8,11–13]. The database allowed us to analyze the structure of immunizing molecules, the generated antibodies, the immunizing epitopes and HLA antigens of the antibodies produced. A potential epitope was deduced by comparing the aminoacid sequences of HLA alleles that are either recognized or not by the alloantiserum.

In this regard, anti-B*14:02 and anti-C*08:01 (detected PrT with low MFI and present on the first donor); anti-C*05:01 and anti-C*06:02 (anti-DSAs in the second donor and detected PTP), and anti-C*07:01, anti-C*18:01 and anti-C*12:02 (non-DSA detected PTP) antibodies share several eplets (9D, 11AVR, 76VRN, and 113YD) (Table 2). The 11AVR eplet is the only one present on all alleles and, consequently, MFIs of all antibodies sharing this eplet increased earlier after transplantation. The location of each epitope is shown on the 3D structure of the HLA antigens (Fig. 1).

Thus, the recognition of the specific 11AVR eplet (in HLA-B*14 and -C*08) in the first transplant modeled the recipienťs antibody response: antibodies that were detected in the second post-transplant, had not been detected or had been detected with a very low MFI in the second pre-transplant. However, the production of *de novo* antibodies against donor HLA-C*05:01 and C*06:02 alleles cannot be discarded. Indeed, eplet 11AVR is also present in other molecules such as HLA-C*15:01 and -C*16:01. The beads coated with these HLA molecules seem to be negative for the post-transplant serum, although their MFI values increased very slightly (Table 1). The variation in antigen density on antibody detection beads or the presence of blocking IgM HLA-specific antibodies that may mask relevant allosensitization could explain those discrepancies. The information on the eplets for class I alleles expressed by the patient is shown in Table 2.

3. Discussion

In general, the involved eplets are easily visible on the top of the molecules adjacent to the bound peptide [2]. Such is the case of the 11AVR eplet we have studied. Immunizing antigens have