

Low median fluorescence intensity could be a nonsafety concept of immunologic risk evaluation in patients with shared molecular eplets in kidney transplantation

Alexandre Bosch ^a, Santiago Llorente ^b, Julio A. Diaz ^a, Gema Salgado ^a, Manuela López ^a, Francisco Boix ^a, Ruth López-Hernández ^a, María J. González-Soriano ^b, José A. Campillo ^{a,c}, María R. Moya-Quiles ^{a,c}, Noelia Perez-Lopez ^a, Alfredo Minguela ^{a,c}, Luisa Jimeno ^b, María R. Álvarez-López ^{a,c}, Manuel Muro ^{a,c,*}

^a Department of Immunology, University Hospital Virgen, Arrixaca, 30120 Murcia, Spain

^b Department of Nephrology, University Hospital Virgen, Arrixaca, 30120 Murcia, Spain

^c Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas, University Hospital Virgen, Arrixaca, 30120 Murcia, Spain

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ABSTRACT

Human leukocyte antigen (HLA) antibodies are usually “epitope” and not “antigen” specific. This work presents an interesting case concerning Luminex median fluorescence intensity (MFI) levels in antibodies considered low risk (<1,000), but producing humoral rejection. These low-titer antibodies could play an important role in transplantation. A 42-year-old woman was retransplanted with a deceased donor with negative complement-dependent cytotoxicity cross-matching. Our patient was pretransplant (PrT) sensitized to HLA antigens (single antigens (SA) = 31%) for 1 previous transplant. Thus, the formerly detected sensitized antigens were A32, A30, A31, cross-reacting group 5C, and DQ3 with a $MFI_{max} \approx 4,127$. In the posttransplantation period (PTP), the patient exhibited important instability in renal function and we detected an increased SA percentage (61%) with $MFI_{max} = 15,029$ (A*32) with other antigens (detected with a low PrT MFI [$<1,000$]) as anti-A*03 ($MFI_{max} = 13,301$) and anti-A*11 ($MFI_{max} = 13,714$) specificities. Anti-A*03 was a donor-specific antibody (DSA). Renal biopsy was compatible with humoral rejection. The patient was pulsed with methylprednisolone, plasmapheresis, and intravenous immunoglobulin without improvement. Thus, we added anti-CD20 and the initial clinical response was highly favorable. Biopsies resulted in suggestive rejection reversion. MFI A*03 DSA decreased to 6,908 and later to $MFI_{max} = 5,505$. After a 6-month PTP, the patient is well with $MFI_{max} = 3,124$. It was possible to define exactly the potential immunizing epitope eplets whose recognition determined the specific antibody production. A*32:01, A*30:01, A*31:01 (detected PrT), A*11:01, and A*03:01 (detected PTP) alleles have several shared eplets (62QE, 70AQS, and 76VGT), with 62QE being the only eplet present on all alleles. In conclusion, low MFI levels in antibodies considered low risk could be important in posttransplant humoral rejection, although the patient's renal function can be restored. Thus, specific shared eplets should always be investigated with respect to previous transplant mismatches.

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1. Introduction

The donor-specific human leukocyte antigen (HLA) antibody (DSA) response of recipients to a renal allograft is not fully understood [1]. Until recently, it has been difficult to detail the evolution of HLA antibodies after transplantation because the methods used to measure HLA antibody levels were not sensitive and cumbersome. Particularly in antibody screening, newer serum screening methods (*i.e.*, Luminex assay) have greatly enhanced the detection

and specificity analysis of anti-HLA antibodies in sensitized patients [2–5].

In this sense, HLA antibodies are usually “epitope” and not “antigen” specific. Patients who lose a graft develop wide antibody patterns specific for the HLA molecules sharing the donor-mismatched epitopes (*i.e.*, cross-reacting groups). Luminex bead technology helps not only in the identification of antibody specificities but also in the identification of these potential antibody epitopes.

Different protocols have recently emerged using B lymphocyte-depleting molecules (anti-CD20), intravenous immunoglobulin (IVIG), and plasmapheresis to prevent and treat humoral rejection [3,6]. Sensitive DSA screening techniques have also emerged, lead-

* Corresponding author.

E-mail address: manuel.muro@carm.es (M. Muro).

ing to better stratification of immunologic risk and better post-transplant detection of humoral rejection development.

2. Case description

This work presents an interesting clinical case concerning Luminex median fluorescence intensity (MFI) levels in antibodies considered low risk (<1,000) [7–9] but producing humoral rejection and sharing specific eplets. These low-titer antibodies could play an important role in transplant outcome.

A 42-year-old woman was retransplanted with a deceased donor. The donor–recipient HLA typings were as follows: recipient (HLA-A*26; B*07,*37; DRB1*10,*15; DQB1*05,*06) and donor (A*02,*03; B*07; DRB1*03,*15; DQB1*02,*06). Our patient was pre-transplant (PrT) sensitized to HLA antigens (single antigens [SA] = 31% by microbead array–Luminex [Labscreen, One Lambda, Canoga Park, CA]) for 1 previous transplant (12 years prior) performed with A32, B35, and DR4 mismatches (first donor: A*26,*32; B*07,*35; DRB1*04,*15; DQB1*03,*06). The presence of antibodies was detected using goat antihuman immunoglobulin G coupled with phycoerythrin. With the Luminex analyzer (LabScan), the reporter fluorescence of each bead was determined and expressed as MFI, which is directly proportional to the amount of antibody bound to the microspheres. MFIs higher than 1,000 are considered positive, as widely reported [3,5,7–9]. MFI_{max} refers to the highest MFI level. All tests were performed as recommended by the manufacturer and as previously published [5,10].

Historically, our patient was only complement-dependent cytotoxicity (CDC) screening positive for A32 antigen. Her last transfusion was performed in 1996 and she has not had any pregnancy. With Luminex technology, we detected additional stable antigens over several years (A32, A30, A31, B35, B53, B72, B51, B52, and DQ3). Thus, the final PrT-detected sensitized antigens were A32, A30, A31, cross-reacting group 5C, and DQ3 with a MFI_{max} ≈ 4,127 (Table 1).

Before transplantation, cross-matching (CM) of the patient's serum and the unseparated donor's lymphocytes (standard protocol in our center) by standard CDC assay was negative.

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

Our center is the exclusive provider of renal transplant follow-up in a large region in the southeast of Spain and performs biopsies of all transplants with indications (dysfunction and/or proteinuria).

During posttransplantation period (PTP) monitoring (15 days PTP), the patient exhibited important instability in renal function

(Cr = 2.1 mg/dL and proteinuria [Pr = 6.5 g/24 hour]) and we detected an increased SA percentage (61%) and MFI level. Hence, increased MFIs in detected PrT antibodies such as anti-A*32 (15,029), anti-A*30 (14,704), and anti-A*31 (11,324), together with other antibodies (undetected or detected with a low PrT MFI [<1,000]) such as anti-A*03 (13,301) and anti-A*11 (13,714), were observed (Table 1). Anti-A*03 was a DSA (present on the second donor). Indeed, we performed posttransplantation (from frozen donor's spleen) CM by CDC and flow cytometry assay with the pre- and posttransplant sera, which were negative in pretransplant and positive in posttransplant. The CM CDC (with extended times for increased sensitivity) and flow cytometry tests were performed as previously published [4,5].

Given these facts, nephrologists indicated a renal biopsy that was compatible with humoral allograft rejection and positive for C4d deposition. The patient was pulsed with methylprednisolone (3 boluses of 500 mg), plasmapheresis (3 sessions/day every 5 days), and IVIG (0.25 g/kg with the last session 1 g/kg) without functional improvement. One week later, the MFI_{max} data post-PP/IVIG did not differ from that of the previous samples.

Thus, we added intravenous anti-CD20 (rituximab, Roche Pharmaceuticals) at 500 mg (2 doses) and the initial clinical response was highly favorable. Biopsies on the 35th PTP (post-anti-CD20 administration) resulted in suggestive rejection reversion (Cr = 1.1 mg/dL and proteinuria [Pr = 2.2 g/24 hours]). MFI A*03 DSA decreased to 6,908 and later to MFI_{max} = 5,505 (Table 1).

After a 6-month PTP, the patient is well with MFI_{max} = 3,124, serologic creatine <1.4 mg/dL, and proteinuria index <100 mg/dL. Renal ultrasound scan also indicates a renal normal graft and renal gammagraphy indicates seemingly good perfusion.

All pre- and posttransplant samples were tested twice and MFI values were reproducible (±200 MFI units). The values presented in Table 1 correspond with the first determination.

Interestingly, it was possible to define exactly the potential immunizing epitope eplets whose recognition determined the specific antibody production. To study this interesting case in depth, high-resolution HLA typings of our recipient and donors were performed, as previously published [5]. Then, to identify the epitope recognized by these alloantibodies, we compared and analyzed the amino acid sequences of the patient's HLA class I molecules with those of the HLA alleles corresponding to antibodies observed pre- and posttransplant using an online database (<http://www.ebi.ac.uk/imgt/hla>; HLA Informatics group section at the Anthony Nolan Web site), Histocheck, HLA Matchmaker, and RasMol version 2.2 (Molecular Graphics Visualisation Tool, Biocomputing Research Unit, Edinburgh, Scotland; <http://www.bernstein-plus-sons.com/software/rasmol/README.html>) [11–13] analyzing the structure of immunizing molecule, the generated antibodies, the immunizing

Table 1
Values of SA Luminex MFI for different alleles in the studied sera

| Allele | Pre-second Tx 0-day MFI | Post-second Tx 15-day MFI | Post-second Tx 35-day MFI | Post-second Tx 49-day MFI | Post-second Tx 180-day MFI |
|-------------|----------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|
| A*32:01 | 4,127 | 15,029 | 7,121 | 6,529 | 4,507 |
| A*30:01 | 1,586 | 14,704 | 6,203 | 5,344 | 3,621 |
| A*31:01 | 1,441 | 11,324 | 5,513 | 3,218 | 2,974 |
| A*03:01 DSA | 989 | 13,301 | 6,908 | 5,508 | 3,124 |
| A*11:01 | 427 | 13,714 | 5,989 | 5,112 | 4,703 |
| A*01:01 | 121 | 827 | 621 | 599 | 333 |
| A*36:01 | 158 | 741 | 426 | 392 | 264 |
| A*74:01 | 136 | 606 | 396 | 378 | 199 |
| B*35:01 | 2,501 | 10,311 | 7,278 | 6,291 | 4,443 |
| B*53:01 | 2,126 | 9,230 | 5,122 | 4,636 | 3,177 |
| B*51:01 | 1,216 | 9,032 | 4,234 | 3,724 | 2,365 |
| B*15:03 | 2,656 | 9,235 | 3,998 | 2,417 | 1,821 |
| B*52:01 | 1,021 | 7,013 | 4,187 | 1,929 | 1,743 |

MFI, median fluorescence intensity; SA, single antigen; Tx, transplant.

Table 2
Different eplets implicated on antibody response in our patient as estimated by Luminex technology

| | 9 | 14 | 56 | 62 | 70 | 76 | 82 | 105 | 113 | 116 | 144 | 149 | 151 | 275 |
|---------|---|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pre-Tx | | | | | | | | | | | | | | |
| A*32:01 | F | | | QE | | ERI | ALR | | | | | | | |
| A*30:01 | S | RS | R | QE | AQS | VGT | | S | YE | H | | | RW | EL |
| A*31:01 | T | | R | QE | | VGT | | S | | | | | | |
| Post-Tx | | | | | | | | | | | | | | |
| A*03:01 | F | | | QE | AQS | VGT | | S | YR | | TKR | AAH | | EL |
| A*11:01 | | | | QE | AQS | VGT | | | YE | | TKR | AAH | HA | EL |

Tx, transplant

epitopes, and HLA antigens of the antibody producer. By comparing the amino acid sequences of a group of HLA-A alleles that are either recognized or not recognized by the alloantiserum, a likely or potential epitope was deduced.

Antibodies to HLA mismatches are specific for epitopes rather than antigens. HLAMatchmaker considers each HLA antigen as a string of eplets that represent key elements of epitopes.

In this sense, anti-A*32:01, A*30:01, and A*31:01 (detected PrT) and anti-A*11:01 and A*03:01 (detected PTP) alleles have several shared eplets (62QE, 70AQS, and 76VGT), with 62QE being the only eplet present on all alleles (Table 2 and Fig. 1).

Thus, recognition of the specific 62QE eplet in the first transplant modeled the antibody response of our renal recipient with undetected antigens (or with a low MFI) in the second pretransplant (A*03 and A*11) and later being detected posttransplantation. However, the production of *de novo* antibodies against an HLA-A*03 donor cannot be ignored. Indeed, the eplet 62QE is also shared by other molecules such as A*01:01, A*36:01, and A*74:01. The beads coated with these HLA molecules seem to be negative with posttransplant serum, although their MFI values increased (Table 1). A possible explanation for these discrepant facts could be the variation in antigen density on antibody detection beads or the presence of blocking immunoglobulin M HLA-specific antibodies that may mask relevant alloantigenization.

3. Discussion

Certain antibodies are specific for single eplets [5], but recent studies have also demonstrated that epitopes defined by eplet pairs always involve 1 nonself eplet and a self eplet shared between the immunizing antigen and the antibody producer [5,14,15]. The activation of self-HLA-specific B cells by a nonself eplet may require that the remainder of the structural epitope of the immunizing

antigen has considerable structural similarity with 1 of the antibody producer's alleles [16].

Generally, the implicated eplets are readily visible at the top of the molecule adjacent to the bound peptide, and many involve contiguous sequences [14], such as the 62QE eplet implicated in this interesting case. A few polymorphisms are visible on the bottom part of the molecule near the cell membrane. Immunizing antigens have mismatched eplets that can form antibody-reactive epitopes with self configurations on the molecular surface [16].

Diverse reports indicate conflicting and disparate values concerning the cutoff MFI value to be considered positive. However, the majority of reports establish the cutoff value as 1,000 or 1,500 [7–9,17–19]. In contrast, a recent report suggests that an MFI value above 300 can be considered positive [20]. However, recently members of the Food and Drug Administration have published an article indicating that solid-phase HLA antibody detection assays were not designed to provide quantifiable data [21]. Our present report appoints an MFI value <1,000 as having clinical relevance on acute rejection development in renal transplantation. These conflicting points should be clarified in future studies.

In conclusion, low MFI levels in antibodies considered low risk could be important in posttransplant humoral rejection, although the patient's renal function can be restored. Thus, alloantibodies can form against single amino acid substitutions and specific shared eplets should always be investigated with respect to previous transplant mismatches and MFI levels. However, our conclusions in the description of an individual case should be taken with caution and do not alter general procedures in transplantation. Patients receiving a graft in the same situation can achieve good renal function.

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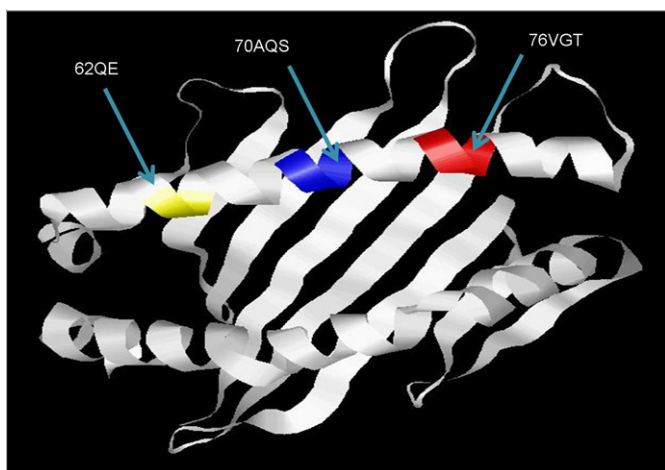


Fig. 1. Implicated alleles have several shared eplets (62QE, 70AQS, and 76VGT), with 62QE being the only eplet present on all alleles.

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