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Shared Molecular Eplet Stimulates Acute Antibody-Mediated Rejection in a Kidney Transplant Recipient With Low-Level Donor-Specific Antibodies: A Case Report

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ABSTRACT

HLA antibodies usually recognize epitopes rather than antigens. This case report reveals that acute antibody-mediated rejection (AMR) that occurred in a kidney transplant recipient with low-level donor-specific antibodies (DSAs) could be explained by shared epitope. A 39-year-old woman received a first kidney transplant from a deceased donor (HLA-DRB1*11:06, *12:02, DRB3*02:02, *03:01). She developed acute AMR confirmed by kidney biopsy on day 4 after transplantation. Antibody testing with pretransplant serum showed anti-DR11 DSA below cutoff level (mean fluorescence intensity [MFI], 702; cutoff >1,000). However, high-level DSAs were detected on day 5 after transplantation (anti-DR11 MFI, 8,531; anti-DR12 MFI, 3,146). We hypothesized that the sharp rise in DSA levels was a result of anamnestic response with donor-antigen sensitization that occurred during pregnancy. High-resolution HLA-DR typing of her husband showed HLA-DRB1*03:01, *15:02:01, DRB3*02:02, DRB5*01:02. No sharing between donor HLAs eliciting reactive antibodies and her husband's HLAs was detected. Nevertheless, we speculated that shared epitope, not antigen, was the cause of allosensitization. To identify the shared epitope recognized by patient's antibodies, we used HLAmatchmaker, a computer algorithm that considers small configurations of polymorphic residues referred to as eplets as essential components of HLA epitopes for analysis. The results showed that 149H, which was the eplet shared by HLA-DRB1*03:01 (from her husband) and DRB1*11:06, DRB1*12:02, DRB3*03:01 (from donor), was the most prevalent eplet on DRB1 reactive alleles in Luminex assay. In conclusion, pretransplant low-level DSAs can induce AMR early after transplantation as a result of shared epitopes with a previous immunizer.

THE antibodies directed against donor HLAs (donor-specific antibodies [DSAs]) represent a major barrier to successful kidney transplantation. Such antibodies are the result of immune response to mismatched HLAs, which can occur after pregnancy (through exposure to paternal nonself antigens in the fetus), blood transfusions, and previous transplantation. The recent development of solid-phase antibody detection assay with single HLA alleles has improved the sensitivity and accuracy for the detection of HLA antibodies [1]. Nevertheless, some patients who had been allosensitized have no detectable DSAs in their sera before transplantation. These patients are at high risk for an anamnestic response occurring in the immediate post-transplantation period.

HLA antibodies react with epitopes on antigenic molecules. It has been shown that patients who experience acute antibody-mediated rejection and/or chronic rejection developed wide antibody patterns specific for the HLA molecules sharing the donor-mismatched epitopes, suggesting that the development of non–donor-specific alloantibodies (NDSAs)

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is due to epitope sharing within HLAs [2,3]. These kidney transplant recipients developed rejections after their second transplant and were initially sensitized to mismatched eplets during the first transplant. A report which described epitope sharing in a sensitized patient without history of transplantation has not yet been published. The present work presents a very interesting case regarding shared epitope sensitization of a first kidney transplant recipient who had very low titer DSA in her pretransplant serum. The sensitization of an epitope shared by previous immunizer (paternal HLA) and the donor resulted in a severe acute antibody-mediated rejection in early posttransplantation.

CASE REPORT

A 42-year-old woman received a first kidney graft from a deceased donor. The donor-recipient HLA typings were as follows: recipient: HLA-A*02, –; B*07, *15:25; DRB1*10, *15; DQB1*05, –; and donor: HLA-A*02, *24; B*07, *46; DRB1*11, *12; DQB1*03, –. The patient's panel reactive antibody (PRA) by Luminex PRA was 66%. Historically, the patient had a blood transfusion 8 years before her transplantation. She had 2 children. The last pregnancy was 10 years earlier.

The anti–human globulin–augmented complement-dependent cytotoxicity crossmatch (AHG-CDC) was negative for T and B cells before transplantation. The presence of HLA antibodies was tested by Labscreen single antigen multiplex solid phase immunoassay (One Lambda, Canoga Park, California) according to the manufacturer's instructions as previously described [4]. Beads with normalized mean fluorescence intensity (MFI) ≥1,000 were considered to be positive. No DSAs were detected in pre-transplantation serum, although NDSAs reactive to DR13, DR14, DR17, and DR18 antigens were detected. Notably, her mean MFI of the reactivities to beads carrying DR11 antigen (donor antigen) was 702, which was below the cutoff level.

The patient received an anti-CD25 monoclonal antibody at induction, followed by tacrolimus, mycophenolate mofetil, and prednisolone. After the transplantation, she exhibited immediate graft function. However, her urine output decreased significantly on day 2 after transplantation. Her serum creatinine level had increased from 3.52 mg/dL on day 0 to 6.45 mg/dL. Ultrasonography showed no hematoma or fluid collection. The renal allograft biopsy on post-transplantation day 4 demonstrated marked transplant glomerulitis (g3), severe peritubular capillaritis (ptc3), and focal C4d staining (C4d2), compatible with acute antibody-mediated rejection (Banff category 2 type II). Luminex single antigen bead assay tested with day 5 post transplantation serum revealed the presence of DSAs directed against DR11 and DR12 antigens. NDSAs reactive to DR8, DR13, DR14, DR17, and DR18 antigens were also demonstrated.

The patient was treated with 6 sessions of plasmapheresis and intravenous immunoglobulin (IVIG), but her renal function did not improve. Therefore, antithymocyte globulin (ATG) was added. The DSA MFI (anti-DR11) decreased to 648 after ATG administration and her urine output increased to 650 mL/d; nevertheless, hemodialysis was required. Unfortunately, a large perinephric fluid collection was found and removed on day 26 after transplantation. The renal allograft biopsy performed on the same day showed unremarkable glomerulitis (g0) and less peritubular capillaritis (ptc2) compared with the previous biopsy. Her urine output increased slowly, and she was discharged after 67 days of hospitalization with the continuation of hemodialysis once a week. Her serum creatinine level at discharge was 4.4 mg/dL.

Table 1. Mismatched HLA-DR Eplets of Previous Immunizer and the Donor for the Patient

Patient's typing: DRB1*10:01,*15:01:01; DRB5*01:01:01		
	No. of mismatched eplets	Mismatched eplets for patient
Mismatched previous immunizer alleles		
DRB1*03:01	13	12STS, 14SEH, 25HRY, 26RY, 26TYD, 31YYFH, 32FHN, 67LK, 71QKG, 73GRDN, 76GDN, 96HV, 149H
DRB1*15:02	0	–
DRB3*02:02	14	12LKS, 14SEH, 26KFE, 32FHN, 40EFD, 67LK, 71QKG, 74QKGQ, 76GDN, 51R, 96HV, 98QS, 104AK, 189S
DRB5*01:02	1	31QGIY
Mismatched donor alleles		
DRB1*11:06	8	12STS, 14SEH, 26TFD, 31YYFY, 57DE, 81HA, 96HV, 149H
DRB1*12:02	8	14GEY, 25YRL, 32FHN, 40EFD, 57VA, 81HA, 96HV, 149H
DRB3*02:02	14	12LKS, 14SEH, 26KFE, 32FHN, 40EFD, 67LK, 71QKG, 74QKGQ, 76GDN, 51R, 96HV, 98QS, 104AK, 189S
DRB3*03:01	15	12LKS, 14SEH, 26KFE, 31LYFH, 32FHN, 40EFD, 57VA, 67LK, 71QKG, 74QKGQ, 76GDN, 96HV, 98QS, 104AK, 149H

The accelerated production of DSAs and NDSAs in a very short period of time after the first kidney transplantation in the patient presented a conundrum. Given the history of sensitization of this patient, we speculated that the original allosensitization had occurred during pregnancies and that the sharp rise in DSA levels was a result of anamnestic response from shared antigens between the previous immunizer and the donor. Nevertheless, this speculation cannot explain why the levels of NDSAs also increased. To explore this hypothesis further, high-resolution HLA DR typings of the patient, her husband, and the donor were performed. The patient was typed as HLA-DRB1*10:01, *15:01:01, DRB5*01:01:01; the donor as HLA-DRB1*11:06, *12:02:01, DRB3*02:02, DRB3*03:01; and the patient's husband (ie, previous immunizer) as HLA-DRB1*03:01, *15:02:01, DRB3*02:02, DRB5*01:02. Although the donor and the previous immunizer shared DRB3*02:02, this antigen sharing could not explain the serum reactivity pattern demonstrated after transplantation by the Luminex single antigen bead assay.

Alloantibodies against HLAs are epitope specific and not antigen specific. The anamnestic response might not be due to sensitization of shared antigens, but of shared epitopes. We then conducted an HLAmatchmaker (www.hlamatchmaker.net) analysis of serum reactivity with Luminex single class II alleles to determine which epitopes, referred to as eplets, were recognized by this patient's antibodies. HLAmatchmaker is a computer algorithm that predicts epitope structure on HLA molecules from stereochemical models of protein antigen-antibody complexes [5]. It is designed to determine HLA compatibility at the epitope level and to analyze serum reactivity for HLA epitope-specific antibodies. The term "eplet"

Table 2. Antibody Reactivity With Single HLA Class II Alleles in the Studied Sera

	MFI			
	Pre-tx serum	Day 5 post-tx serum	Immunizer eplets on reactive alleles	
DRB1*03:01	1487	12701	12STS, 26TYD, 31YYFH, 149H	
DRB1*03:02	1033	9635	12STS, 26TFE, 31YYFH, 149H	
DRB1*08:01	766	8250	14GEY, 26TFD, 31YYFY, 149H	
DRB1*11:01	616	7741	12STS, 26TFD, 31YYFY, 57DE, 149H	
DRB1*11:04	789	9322	12STS, 26TFD, 31YYFY, 57DE, 149H	
DRB1*12:01	50	2861	14GEY, 25YRL, 149H	
DRB1*12:02	47	3432	14GEY, 25YRL, 149H	Donor allele
DRB1*13:01	987	9191	12STS, 26TFD, 31YYFH, 149H	
DRB1*13:03	1245	12922	12STS, 26TFD, 31YYFH, 149H	
DRB1*14:01	1349	11222	12STS, 26TFD, 31YYFH, 149H	
DRB1*14:02	519	6830	12STS, 31YYFH, 149H	
DRB3*02:02	26	1261	12LKS, 74QKGQ, 51R	Donor allele
DRB3*03:01	72	81	149H	Donor allele
Negative control	146	60		
Positive control	8847	7687		
Mean MFI of self alleles	138.75	51.75		

Abbreviations: MFI, mean fluorescence intensity; tx, transplantation.

refers to polymorphic HLA residues within 3 Å of each surface-exposed polymorphic position in the molecular sequence. Notations of eplets consist of amino acid sequence numbers and polymorphic residue descriptions with standard single letter codes.

The results showed that the previous immunizer had 3 mismatched alleles: DRB3*02:02 with 14 mismatched eplets, followed by DRB1*03:01 (13 mismatched eplets) and DRB5*01:02 (1 mismatched eplet). HLA-DRB1*15:02 in the previous immunizer was a structural match for the patient. The donor had 4 mismatched alleles: DRB3*03:01 with 15 mismatched eplets, DRB3*02:02 (14 mismatched eplets), DRB1*11:06 and DRB1*12:02 (each with 8 mismatched eplets). Several eplets were shared between the previous immunizer and the donor (Table 1).

This study aimed to explain the accelerated production of DSAs and NDSAs after transplantation in the patient who had no detectable DSAs before transplantation. The MFI values of reactive alleles in post-transplantation serum compared with that of pre-transplantation serum are presented in Table 2. The reactivity against DRB1*12:02 (donor's allele) showed an MFI value of 3,432. This allele shared the 149H eplet with DRB1*03:01 in the previous immunizer. Moreover, 9 reactive alleles, DRB1*03:02, DRB1*08:01, DRB1*11:01, DRB1*11:04, DRB1*12:01, DRB1*13:01, DRB1*13:03, DRB1*14:01, and DRB1*14:02, shared 149H with DRB1*12:02. These findings suggested that the production of DSAs (reactivities to DR11 and DR12 antigens) and NDSAs (reactivities to DR8, DR13, DR14, DR17, and DR18 antigens) reflected the presence of a 149H-specific antibody originally sensitized by the DRB1*03:01 mismatched from the previous immunizer. The kidney graft from the donor with DRB1*12:02, DRB1*11:06, and DRB3*03:01 shared 149H with the previous immunizer subsequently induced the increased production of 149H-specific antibody.

DISCUSSION

This report illustrates the importance of antibodies specific for an epitope in organ transplantation. The epitope-specific antibody can explain the serum antibody reactivity patterns found with Luminex single antigen bead panel. In our case, sensitization to 149H was initially induced during previous pregnancies by DRB1*03:01, a mismatched allele of the fetopaternal HLA. Once alloantigen exposure occurred via pregnancy, B cells were activated in a T-cell–dependent manner. After T-dependent activation, B cells can undergo 2 different fates; either they can migrate out of B-cell follicles and become responsible for the early production of low-affinity antibody, or they enter a germinal center where they undergo somatic hypermutation and class switch recombination [6]. Mutated clones with higher affinity for antigen are positively selected and differentiate into memory B cells or plasma cells. A small proportion of plasma cells migrate to the bone marrow, where they become long-lived plasma cells. Survival of long-lived plasma cells is regulated through competition for limited survival niches. Once in survival niches, the persistence of long-lived plasma cells does not require the presence of antigen. On reexposure to the same alloantigen, memory B cells expand rapidly, resulting in a peak of plasma cell clones in peripheral blood within 7 days [7]. A sharp rise in post-transplantation antibodies that reacted with 149H-carrying DRB1 alleles in the Luminex panel in our case was possibly due to this anamnestic response. This highlights the concept of epitope sharing within HLA antigens and suggests that the development of NDSAs is a result of a reaction against an epitope shared by previous immunizer and the donor.

Early post-transplantation antibody-mediated rejection experienced by a patient without pre-transplantation DSAs, as shown in the present case report, challenges the concept of low immunologic risk in kidney transplant recipients with negative complement-dependent cytotoxicity crossmatch (CDC-XM) and negative DSAs. The failure to identify epitope-specific antibody before transplantation in our patient was due to very low reactivity to beads carrying DR11 and DR12 (donor antigens). Remarkably, the reactivity to DRB1*03:01 (anti-DR17) and DRB1*03:02 (anti-DR18) in

pre-transplantation serum was higher than the cutoff level. Because DR17 and DR18 were not donor antigens, these antibodies were neglected. These finding suggest that using the MFI cutoff value of 1,000 might not be suitable. Nevertheless, this cutoff value has been widely used in many studies [2,8,9]. Furthermore, a study of 113 patients by Aubert et al showed that DSA with MFI <2,000 was not associated with short-term rejection events [10]. A study by Morris et al demonstrated that DSA with MFI <2,000 does not result in a positive CDC-XM and may be not a contraindication for transplantation [11]. Conversely, if we lowered the cutoff level to MFI ≥500, the clinically important DSA (anti-DR11) would have been detected in the pre-transplantation serum in our patient. At present, it is still unclear which cutoff level is most appropriate for the determination of clinically relevant DSA.

The determination of epitope-specific antibodies in sensitized patients has an important implication for organ transplant allocation. An epitope analysis of serum antibody reactivity of sensitized patients would be beneficial for the identification of permissible HLA antigens. It would be more meaningful to evaluate the degree of HLA compatibility based on epitopes rather than HLA antigens. Indeed, Eurotransplant has implemented HLAmatchmaker, an epitope-matching algorithm, in the Acceptable Mismatch Program to identify donors for highly sensitized patients and has demonstrated reduced waiting time and excellent graft survivals [12,13].

In summary, early post-transplantation antibody response to an epitope shared by previous immunizer and the donor could induce severe antibody-mediated rejection even though the patient had negative or very low level of DSA in pre-transplantation serum. With the use of the computer algorithm analysis, determination of epitope-specific antibody from serum reactivity pattern with single antigen assay greatly enhances an understanding of not only the post-transplantation production of DSAs, but also the production of NDSAs against antigens which the patient has never been exposed to.

REFERENCES

[1] Taylor CJ, Kosmoliaptsis V, Summers DM, et al. Back to the future: application of contemporary technology to long-standing questions about the clinical relevance of human leukocyte antigen–specific alloantibodies in renal transplantation. Hum Immunol 2009;70:563–8.

[2] 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.

[3] Thaunat O, Hanf W, Dubois V, et al. Chronic humoral rejection mediated by anti–HLA-DP alloantibodies: insights into the role of epitope sharing in donor-specific and nondonor specific alloantibodies generation. Transpl Immunol 2009;20:209–11.

[4] Thammanichanond D, Ingsathit A, Mongkolsuk T, et al. Pretransplant donor specific antibody and its clinical significance in kidney transplantation. Asian Pac J Allergy Immunol 2012;30:48–54.

[5] Duquesnoy RJ. Antibody-reactive epitope determination with HLAmatchmaker and its clinical applications. Tissue Antigens 2011;77:525–34.

[6] Clatworthy MR, Espeli M, Torpey N, et al. The generation and maintenance of serum alloantibody. Curr Opin Immunol 2010;22:669–81.

[7] Lanzavecchia A, Sallusto F. Human B cell memory. Curr Opin Immunol 2009;21:298–304.

[8] Billen EV, Voorter CE, Christiaans MH, et al. Luminex donor-specific crossmatches. Tissue Antigens 2008;71:507–13.

[9] Zoet YM, Brand-Schaaf SH, Roelen DL, et al. Challenging the golden standard in defining donor-specific antibodies: does the solid phase assay meet the expectations? Tissue Antigens 2011;77:225–8.

[10] Aubert V, Venetz JP, Pantaleo G, et al. Low levels of human leukocyte antigen donor–specific antibodies detected by solid phase assay before transplantation are frequently clinically irrelevant. Hum Immunol 2009;70:580–3.

[11] Morris GP, Phelan DL, Jendrisak MD, et al. Virtual crossmatch by identification of donor-specific antihuman leukocyte antigen antibodies by solid-phase immunoassay: a 30-month analysis in living donor kidney transplantation. Hum Immunol 2010;71:268–73.

[12] Claas FH, Witvliet MD, Duquesnoy RJ, et al. The acceptable mismatch program as a fast tool for highly sensitized patients awaiting a cadaveric kidney transplantation: short waiting time and excellent graft outcome. Transplantation 2004;78:190–3.

[13] Doxiadis II, Duquesnoy RJ, Claas FH. Extending options for highly sensitized patients to receive a suitable kidney graft. Curr Opin Immunol 2005;17:536–40.