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Successful kidney transplantation after desensitization in a patient with positive flow crossmatching and donor-specific anti-HLA-DP antibody

A Case report

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Abstract

Background: Traditionally, the presence of antibodies against human leukocyte antigen (HLA)-C and DP was considered to be associated with only a low risk of antibody-mediated rejection (ABMR) in kidney transplantation (KT), because the antigenicities of these proteins are weak. However, the clinical effects of HLA-C and -DP donor-specific HLA antibodies (DSHAs) have recently been reevaluated.

Methods: Here, we report the case of a retransplant patient with positive flow cytometry crossmatch (FCXM) and high level of HLA-DP DSHA who was desensitized using rituximab, plasmapheresis, and intravenous immunoglobulin.

Results: The epitope-based antibody reactivity was identified that the positive B-cell FCXM in our patient was attributable to the specific epitope. The patient underwent a successful retransplantation and has continued to do well for 10 month after KT.

Conclusion: If an HLA-DP DSHA is present, it is important to detect any mismatched HLA-DP epitope pretransplantation and to monitor HLA-DP levels carefully. According to previous reports, anti-HLA-DP DSHA can induce ABMR soon after transplantation, but such ABMR can be prevented by pretransplantation desensitization and careful monitoring of DSHA levels.

Abbreviations: ABMR = antibody-mediated rejection, CDC-XM = complement-dependent cytotoxicity crossmatching, CMR = cell-mediated rejection, DSHA = donor-specific HLA antibody, FCXM = flow cytometry crossmatch, HLA = human leukocyte antigen, HSCT = hematopoietic stem cell transplantation, HVR = hypervariable region, KT = kidney transplantation, MFI = mean fluorescence intensity, SAB = single-antigen bead.

Keywords: desensitization, donor-specific antibody, HLA-DP antibody, kidney transplantation

1. Introduction

Despite immunological advances in transplantation, antibody-mediated rejection (ABMR) remains the major cause of graft failure after kidney transplantation (KT).[1] The presence of donor-specific anti-human leukocyte antigen (HLA) antibodies (DSHA) increases the risk of ABMR after KT.[2] Luminex technology facilitates sensitive detection of anti-HLA antibodies. However, most clinical studies have focused on antibodies against class I HLA-A and -B and class II HLA-DR and -DQ rather than against class I HLA-C and class II HLA-DP. The clinical implications of anti-HLA-C and -DP antibodies in the context of transplantation are less significant because these antigens are expressed at lower levels than other HLA antigens. Although the levels of mRNAs encoding HLA-C proteins are similar to those encoding HLA-A and-B proteins, but HLA-C expression levels on cell surfaces are much lower than those of HLA-A and -B.[3] Additionally, HLA-DP is expressed at a lower level than HLA-DR on the endothelial surface of the normal kidney.[4]

However, the clinical relevance of anti-HLA-C and -DP DSHAs has recently been reevaluated; it likely that these antibodies can, in fact, trigger acute ABMR.[5] In a previous report, desensitization after KT was unable to overcome the ABMR because of the existence of preformed HLA-DP DSHA.[6] Here, we report a case of successful transplantation after desensitization of a patient who expressed a high level of anti-HLA-DP DSHA and positive flow cytometry crossmatch (FCXM) results. We also review the epitope-matching analysis based on previous cases.

2. Case review

A 26-year-old female with end-stage renal disease caused by IgA nephropathy received her 1st kidney transplant from her mother

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in 2008. HLA typing assays detecting the HLA-A, -B, DRB1, and DQB1 loci were performed using Luminex technology and LIFECODES HLA SSO typing kits (Immucor Transplant Technology, Stamford, CT). HLA-DPB1 typing was achieved by direct sequencing of exons 2, 3, and 4 of *HLA-DPB1*. The Luminex single-antigen bead (SAB) assay (LIFECODES LSA Classes I and II, Immucor Transplant Technology) was performed to explore DSHA status. Complement-dependent cytotoxicity crossmatching (CDC-XM) and FCXM of T- and B-cells were performed before transplantation. The informed consent was given from the patient and the ethical approval was waived for case report.

At the time of the 1st KT, 3 antigen mismatches (HLA-A31, -B75, and -DPw5, Table 1) were evident. The CDC-XM and FCXM tests were both negative, and no anti-HLA antibodies were detected by the SAB assay. The patient underwent induction therapy with baxiliximab (Simulect), followed by tacrolimus (Tacrobell), mycophenolate sodium (Myfortic), and prednisolone (Solondo).

No clinical event was noted for 2 years after the 1st KT. However, the patient developed a urinary tract infection 2 years after the 1st KT, and her serum creatinine level increased to 1.75 mg/dL. Biopsy of the allograft kidney revealed Banff IB/IIA acute cell-mediated rejection (CMR). She underwent steroid pulse therapy and received antithymocyte globulin, but CMR was diagnosed on 4 occasions (in the absence of DSHA) from June 2010 to June 2012. However, the graft function continued to decline. One year later, her serum creatinine level had increased to 3.65 mg/dL and DSHA to B75, and DPB1*05 appeared; the mean fluorescence intensities (MFIs) were 2617 and 8556, respectively, according to the SAB assay. A 5th kidney biopsy was performed, and she was diagnosed with C4d-negative ABMR accompanied by glomerulitis (g1) and peritubular capillaritis (ptc1). One month later, a 6th biopsy was performed, and acute ABMR (g2, ptc1) associated with C4d-positivity was diagnosed (Fig. 1). After the diagnosis of ABMR, she underwent plasmapheresis featuring intravenous immunoglobulin therapy. However, the graft function continued to decline, and her serum creatinine level increased to 5.78 mg/dL.

She was evaluated in terms of retransplantation from 2 candidate donors and underwent a 2nd KT (from her older sister; candidate donor 1) in May 2015; the allograft kidney was mismatched at HLA-A, -B, -DR, and -DP. During pretransplant evaluation, the CDC-XM status was negative, but both T- and B-cell FCXM were positive (Table 1). Anti-HLA-B44, -DPB1*05, and -DPB1*19 DSHAs were present, as revealed by the SAB assay. The Luminex C1q assay was also performed at this time, but it was negative. The MFI values of each DSHA were 1844 (HLA-B44), 14,454 (-DPB1*05), and 11,760 (-DPB1*19).

The hypervariable region (HVR) of *HLA-DPB1* differed between the recipient and donor at amino acids 84 to 87 of exon 2. After the 1st KT, only anti-HLA-DP antibodies were evident in the class II PRA assay. The epitope triggering antibody production was identified using LIFECODES Match It Antibody software version 1.2.1 (Immucor Transplant Technology) as 84 DEAV (donor-specific) (Table 1). The patient had both class I and II DSHAs to candidate donor 1 but only a class II DSHA to candidate donor 2. However, positive B-cell FCXM reactions to both candidate donors were evident. Therefore, the recipient HLA-DPB1*05 DSHA reacted with the donor-specific HLA-DP antigen of both candidate donors.

The patient underwent desensitization therapy prior to the 2nd KT. She was prescribed rituximab at day 7, and underwent 4

Table 1

Histocompatibility test data from the recipient and donors.

	HLA typing	HVR of HLA-DP at 84–87 AA	T-FCXM (MCS)	B-FCXM (MCS)	Class I DSHA (MFI)	Class II DSHA (MFI)
Recipient	A2, A11, B60, B48, DR8, DR14, DQ6, DQ5, DPB1*02, DPB1*02	GGPM				
Donor of 1st transplant	A2, A31, B75, B48, DR8, DR14, DQ6, DQ5, DPB1*02, DPB1*05	GGPM DEAV	NT	NT	B75 (1322)[†]	DPB1*05 (14,454)[‡]
Candidate donor 01 (2nd transplant)	A2, A33, B44, B48, DR13, DR14, DQ6, DQ6, DPB1*05, DPB1*19	DEAV	Positive (129)	Positive (401)	B44 (1884)[‡]	DPB1*05 (14,454), DPB1*19 (11,760)[‡]
Candidate donor 02	A11, A24, B48, B51, DR4, DR4, DQ4, DQ4, DPB1*05, DPB1*48	DEAV	Negative	Positive (355)	None	DPB1*05 (14,454)[‡]

MCS > 77 was considered to be a positive FCXM. The Luminex single-panel reactive antibody assay revealed HLA-B antibodies of class I and HLA-DP antibodies of class II, without any other HLA antibodies. A = alanine, AA = amino acid, D = aspartic acid, DSHA = donor-specific HLA antibody, E = glutamic acid, FCXM = flow cytometry crossmatch, G = glycine, HLA = human leukocyte antigen, HVR = hypervariable region, M = methionine, MCS = median channel shift, MFI = mean fluorescence intensity, NT = not tested, P = proline, V = valine.

[†] The 4-fold serum dilutions exhibited a significantly increased MFI and a prozone effect of HLA-B75 antibody.

[‡] The 4-fold serum dilutions exhibited no prozone effects (data not shown).

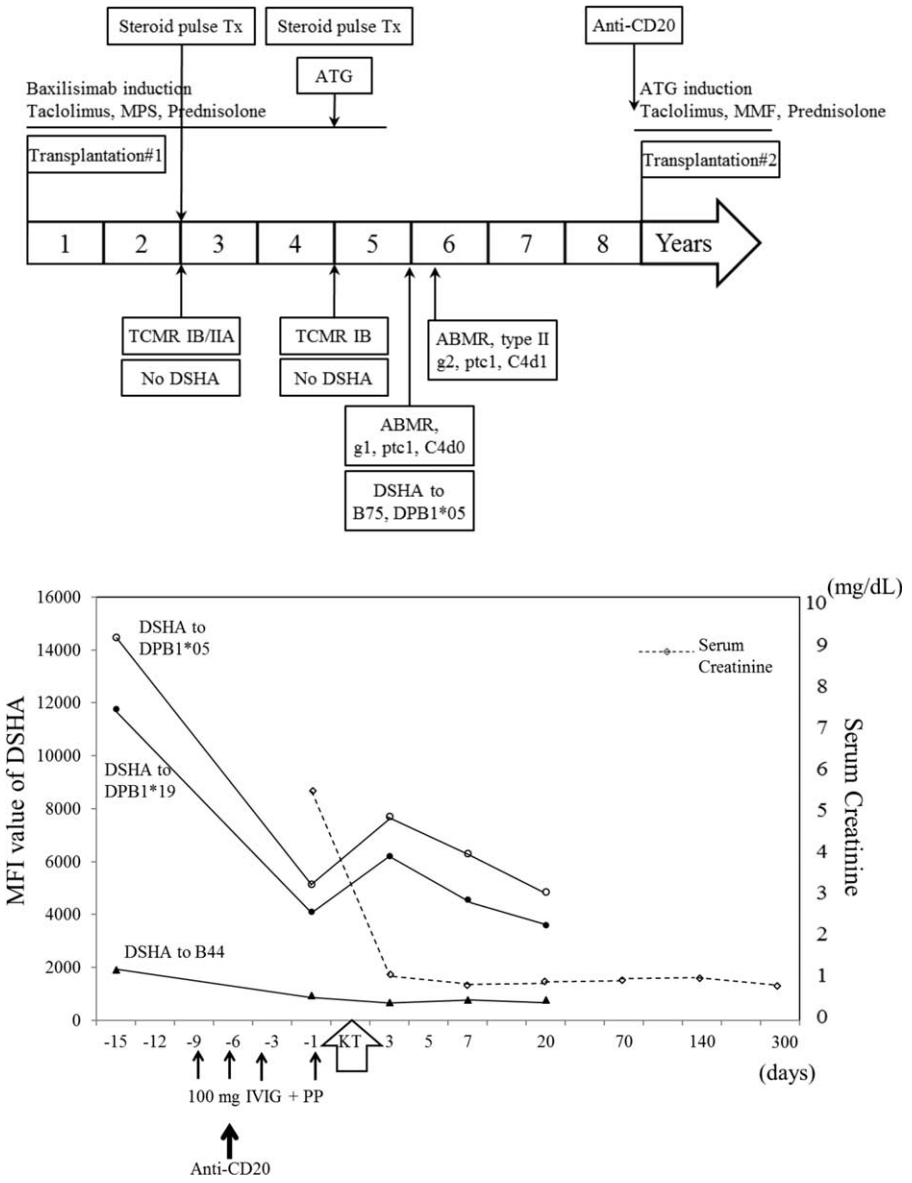

Figure 1. Flow chart of the clinical course after the 1st KT showing the levels of serum creatinine and DSHAs. The initial MFI values for each type of DSHA were -B44 (1884), -DPB1*05 (14,454), and -DPB1*19 (11,760). After desensitization, the MFI values for each type of DSHA decreased to HLA-B44 (914), -DPB1*05 (5,135), and -DPB1*19 (4,093). The MFI values for each type of DSHA were monitored at 3, 7, and 20 days after KT and were HLA-B44 (650), -DPB1*05 (7698), and -DPB1*19 (6180); HLA-B44 (747), -DPB1*05 (6295), and -DPB1*19 (4549); and HLA-B44 (739), -DPB1*05 (4838), and -DPB1*19 (3590), respectively. ABMR = antibody-mediated rejection, ATG = antithymocyte globulin, C4d0 = negative C4d staining, C4d1 = minimally positive C4d staining, DSHA = donor-specific HLA antibody, g = glomerulitis, HLA = human leukocyte antigen, IVIG = intravenous immunoglobulin, KT = kidney transplantation, MFI = mean fluorescence intensity, MMF = mycophenolate mofetil, MPS = mycophenolate sodium, ptc = peritubular capillaritis, TCMR = T cell-mediated rejection.

plasmaphereses using a total of 100 mg/kg intravenous immunoglobulin. After desensitization, the MFI values of the DSHAs fell to 914 (HLA-B44), 5135 (-DPB1*05), and 4093 (-DPB1*19). The T-cell FCXM status was negative prior to KT.

After transplantation, immunosuppression was induced with antithymocyte globulin and maintained with tacrolimus (Tacrobell), mycophenolated mofetil (MMF, Myrept), and prednisolone (Solondo). She was capable of immediate urination and the serum creatinine level stabilized at 0.97 mg/dL 10 months after the 2nd KT. The MFIs of the DSHAs were measured 3, 7, and 20 days after KT, and were 650 (HLA-B44), 7698 (-DPB1*05), and 6180 (-DPB1*19); 747 (HLA-B44), 6295 (-DPB1*05), and 4549 (-DPB1*19); and 739 (HLA-B44), 4838 (-DPB1*05), and 3590 (-DPB1*19), respectively (Fig. 1).

3. Discussion

HLA-DP antigens have been considered to be minimally immunogenic but the incidence of development of anti-HLA-DP antibodies after KT was 8% to 45% in previous reports.[7,8] Development of anti-HLA-DP antibodies were associated with transplantation rather than pregnancy or transfusion events.[5] In our patient, anti-HLA-DP antibodies developed after HLA-DR/DQ-matched transplantation. The patient developed primary

CMR, and DSHA levels gradually increased. Thus, anti-HLA-DP antibodies can develop after KT associated with an HLA-DP mismatch regardless of the presence of ABMR.

DSHAs to HLA-DP target immunogenic epitopes in donor-specific HVRs.[7] The clinical importance of matching immunogenic HLA-DP epitopes in KT and in patients undergoing hematopoietic stem cell transplantation (HSCT) has been emphasized.[7,9,10] However, the epitope-matching concept differs between HSCT and KT. T-cell epitope-matching at HLA-DPB1 is recommended prior to HSCT, but HVR matching of HLA-DP is recommended before KT.[7,9] The HVRs of *HLA-DPB1* are 6 in number, termed HVR-A to -F; about 50% of antibodies target HVR-F (84 DEAV).[7,11,12] Only 7 epitopes (35 FC, 56 E, 56 E^{DR11}, 56EE, 57 D, 84 DEAV, and 85 GPM) of HLA-DP exhibit confirmed antibody reactivity (according to the HLA epitope registry), and 3 HVRs (HVR-B, -C, and -F) are included in the "confirmed epitopes" database (http://www.epregistry.ufpi.br/index/databases/database/DP/, 2015.06.10). Previously, epitope-based matching was thought to be more important than allelic matching of HLA-DP antigens prior to KT.[10] Both our current results and earlier data support the importance of epitope-based matching of HLA-DP antigens (Table 2). The epitope-based antibody reactivity was identified in our patient and the positive B-cell FCXM was attributable to the epitope 84 DEAV.

When using Luminex technology, antibody strength may be overestimated because of differences in the extent of antigenic expression between cells and microbeads. In particular, the MFI value of an anti-HLA-DPB antibody may differ from that of an actual HLA/anti-HLA-DPB reaction.[16] Therefore, the clinical significance of anti-HLA antibodies cannot be estimated using only the Luminex assay.[17] Our patient exhibited a positive B-cell FCXM but a negative CDC-XM.

HLA-DP antigens are known to be associated with a reduced immunological risk of rejection when the CDC-XM status is negative.[17] However, any correlation between ABMR and positive XM data in terms of HLA-DP DSHA remains controversial. Positive CDC or FCXM status has been reported to be associated with ABMR.[6,17,18] However, 1 recent report showed that DSHA to HLA-DP could trigger ABMR shortly after KT in the absence of XM positivity.[14] Also, ABMR can be induced by an antibody reaction to either HLA-DPA or -DPB.[19] In addition, positive B cell FCXM status alone (thus combined with negative CDC-XM status) can trigger both early and late allograft failure.[20] Recent data have shown that HLA-DP DSHA are more relevant in the context of acute ABMR and a positive FCXM status than are DSHA to HLA-A, B, DR, or DQ.[21] Therefore, the positive B cell FCXM status was a poor prognostic marker after transplantation in our patient.

In our present case, the patient underwent desensitization before transplantation, because she was positive for FCXM and exhibited high-level DSHA associated with HLA-DP. After transplantation, the patient was stable, thus without ABMR, for 10 months. According to previous reports, anti-HLA-DP DSHA can induce ABMR soon after transplantation,[13] but such ABMR can be prevented by pretransplantation desensitization and careful monitoring of DSHA levels, as in our case. However, the long-term clinical impact of anti-HLA-DP DSHA requires further evaluation.

In conclusion, HLA-DP antibodies usually develop in the posttransplant status and are of clinical significance. Therefore, HLA-DP DSHA levels should be evaluated, especially if retransplantation is planned. It is important to identify

Table 2

Epitopes mismatched to the donor-specific HLA-DP and clinical courses after transplantation as described in previous reports.

Recipient HLA-DP	Donor HLA-DP	DSHA (MFI)		Mismatched donor epitope†	Crossmatching	ABMR onset after KT	Reference
		Initial	After desensitization				
DPB1*01:01, DPB1*04:02	DPB1*04:01, DPB1*20:01	NA	NA	11L, 35FA, **56ED**, 64DL	Positive B-FCXM	2 months	[6]
DPB1*03:01, DPB1*14:01	DPB1*02:01, DPB1*04:01	NA	NA	**56EE**, 65ER	Positive B-FCXM	12 days	[6]
DPB1*04:01, DPB1*04:01	DPB1*01:01, DPB1*02:01	DPB1*01:01 (9280)	DPB1*01:01 (5666)	8V, 35YA, 76V, **84DEAV**	Positive B-CDC and B-FCXM	4 weeks	[13]
DPB1*02:01, DPB1*04:01	DPB1*04:01, DPB1*03:01/05:02	DPB1*03 (13,216), DPB1*05 (12,453)‡	DPB1*03 (5894), DPB1*05 (6892)‡	8V, 35LV, 55EA, 56ED, 64DL, 76V, **84DEAV**	Positive B-CDC and B-FCXM	11 days	[13]
DPB1*02:01, DPB1*04:01	DPB1*01:01, DPB1*04:02	DPB1*01:01 (16,291)	NA	8V, 35YA, 76V, **84DEAV**	Negative FCXM	11 days	[14]
DPB1*02:01, DPB1*04:01	DPB1*10:01, DPB1*04:01	DPB1*10:01 (1976)	NA	8V, 11L, 76V, **84DEAV**	NA	3.5 years due to de novo HLA-DP DSHA	[15]
DPB1*02:01, DPB1*02:01	DPB1*05:01, DPB1*19:01	DPB1*05 (14,454), DPB1*19 (11,760)	DPB1*05 (5135), DPB1*19 (4093)	35LV, 55EAE, 65KR, 76I, **84DEAV**	Negative B-CDC and Positive B-FCXM	No rejection to 10 months	Present case

A = alanine, ABMR = antibody-mediated rejection, CDC = complement-dependent cytotoxicity, D = aspartic acid, DSHA = donor-specific HLA antibody, E = glutamic acid, F = phenylalanine, FCXM = flow cytometry crossmatching, HLA = human leukocyte antigen, I = isoleucine, K = lysine, KT = kidney transplantation, L = leucine, MFI = mean fluorescence intensity, NA = not applicable, R = arginine, V = valine, Y = tyrosine.

† Mismatched donor epitopes were evaluated using the HLAMatchmaker program. Confirmed epitopes are indicated in bold.

‡ These antibody levels were detected in 100-fold-diluted sera.

mismatched HLA-DP epitopes pretransplantation and to carefully monitor anti-HLA-DP antibody levels. Such donor-specific epitope antibodies can induce ABMR shortly after transplantation. However, acute ABMR developing shortly after transplantation can be prevented by pretransplant desensitization accompanied by close monitoring.

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