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Two Cases of Antibody-Mediated Rejection Following Kidney Transplantation due to *HLA-DQB1* Allele-Specific and DQ Alpha Protein-Specific HLA Antibodies

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Dear Editor,

The HLA-A, HLA-B, and HLA-DR loci have been used for transplant candidate matching. Recent studies using single antigen bead-based assays (SABAs) have revealed that HLA-DQ donor-specific HLA antibodies (DSHAs) are associated with inferior allograft outcomes [1, 2]. Therefore, donor HLA-DQ typing prior to transplantation is now thought to be highly important.

HLA-DQ antigen typing is determined by *HLA-DQB1* genotyping. The HLA-DQ antigen is composed of two alpha protein domains, coded by the *DQA1* gene, and two beta protein domains, coded by the *DQB1* gene. HLA-DQ antigen Luminex SABA beads are coated with both DQ alpha and DQ beta proteins. Therefore, DQ alpha proteins are taken into account in the interpretation of HLA-DQ antibody reactions. However, the clinical significance of DQ alpha-specific DSHA is questionable. In addition, although the clinical significance of allele-specific DSHAs has been established, the routine application of high-resolution HLA genotyping remains controversial [3-5].

We report two representative cases of antibody-mediated rejection (ABMR) following kidney transplantation (KT) due to *HLA-DQB1* allele-specific DSHA and DQ alpha protein-specific DSHA.

HLA typing assays for detecting the *HLA-A, -B, -DRB1*, and *-DQB1* loci were performed by using Luminex technology and LIFECODES HLA SSO typing kits (Immucor Transplant Technology, Stamford, CT, USA). High-resolution *HLA-DQB1* and *DQA1* typing results were achieved through direct sequencing of exons 2, 3 of the *HLA-DQB1* gene and exons 1, 2, 3, 4 of the *HLA-DQA1* gene, using the ABI PRISM 3100 Genetic analyser (Applied Biosystems, Hitachi, Japan). SABAs were performed by using two commercially available Luminex assays (LIFECODES Single Antigen Class I/II [Immucor Transplant Technology] and LABScreen Single Antigen Class I and II [One Lambda, Canoga Park, CA, USA]) and the C1q assay (C1q Screen; One Lambda) was performed to determine DSHA status.

CASE 1

A 21-yr-old woman with end-stage renal disease caused by lupus nephritis received a KT from her mother with 0% calculated panel reactive antibody (PRA) in February 2010. Four years fol-

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lowing the KT, serum creatinine levels increased to 2.32 mg/dL and a biopsy of the allograft kidney revealed active ABMR with diffusely positive deposition of C4d, g3 (glomerulitis, score 3), and ptc3 (peritubular capillaritis, score 3). At the time of rejection, only HLA-DQ6 class II HLA antibodies were identified by SABA. No HLA-DQ mismatch was revealed by antigen-level typing. Therefore, high-resolution *HLA-DQB1* and *DQA1* typing were performed and an *HLA-DQB1*06:01* allele-specific DSHA was identified. The C1q assay revealed positive C1q fixing for *HLA-DQB1*06:01* DSHA (Table 1).

Table 1. HLA typing and antibody reaction results for case 1 (*HLA-DQB1* allele-specific DSHA)

HLA typing	*A*	*B*	*C*	*DRB1*	*DQB1*	*DQA1*
Recipient	11, 26	44, 62	05, 09	04, 13	04:01 (DQ4), 06:03 (DQ6)	01:03, 03:03
Donor (mother)	11, 26	39, 44	05, 07	08, 13	06:01 (DQ6), 06:03 (DQ6)	01:03, 01:03
Antibody reactions by SABA *HLA-DQB1* and *-DQA1* specificity of HLA-DQ beads					Raw MFI from Immucor	Baseline MFI from One Lambda	Baseline MFI for C1q assay
*DQB1*06:01 (DQ6)-DQA1*01:03*					11,941	7,111	14,633
*DQB1*06:01 (DQ6)-DQA1*02:01*					11,827	NA	NA
*DQB1*06:01 (DQ6)- DQA1*01:04*					10,761	NA	NA
*DQB1*06:09 (DQ6)- DQA1*01:02*					NA	6,155	0
*DQB1*06:02 (DQ6)- DQA1*01:01*					NA	314	0
*DQB1*06:03 (DQ6)- DQA1*01:03*					NA	0	0
*DQB1*06:04 (DQ6)- DQA1*01:02*					3,233	591	0

Abbreviations: DSHA, donor-specific HLA antibody; SABA, single antigen bead assay; MFI, mean fluorescence intensity; NA, not applicable.

Table 2. HLA typing and antibody reaction results for case 2 (*HLA-DQA1*-specific DSHA)

HLA typing	*A*	*B*	*DRB1*	*DQB1*	*DQA1*
Recipient	02, 33	48, 61	12, 14	03:01 (DQ7), 05:03 (DQ5)	01:04, 05:08
Donor (mother)	02, 26	48, 55	12, 14	03:01 (DQ7), 05:03 (DQ5)	01:04, 06:01
Antibody reactions by SABA *HLA-DQB1* and *-DQA1* specificity of HLA-DQ beads				Raw MFI from Immucor	Baseline MFI from One Lambda
*DQB1*02:01 (DQ2)-DQA1*04:01*				NA	3,867
*DQB1*03:03 (DQ9)-DQA1*04:01*				3,004	NA
*DQB1*04:02 (DQ4)-DQA1*04:01*				2,833	5,219
*DQB1*04:01 (DQ4)-DQA1*04:01*				2,599	NA
*DQB1*02:01 (DQ2)-DQA1*05:01*				1,323	4,626
*DQB1*02:02 (DQ2)-DQA1*05:01*				1,450	NA
*DQB1*04:01 (DQ4)-DQA1*05:01*				2,003	NA
*DQB1*03:01 (DQ7)-DQA1*02:01*				NA	Negative
*DQB1*03:01 (DQ7)-DQA1*03:01*				NA	Negative
*DQB1*03:01 (DQ7)-DQA1*03:02*				Negative	NA
*DQB1*03:01 (DQ7)-DQA1*05:01*				2,993	NA
*DQB1*03:01 (DQ7)-DQA1*05:03*				NA	4,627
*DQB1*03:01 (DQ7)-DQA1*05:05*				NA	3,174
*DQB1*03:01 (DQ7)-DQA1*06:01*				2,538	4,591
*DQB1*03:03 (DQ9)-DQA1*06:01*				2,386	NA
*DQB1*04:02 (DQ4)-DQA1*06:01*				2,075	NA

Abbreviations: see Table 1.

CASE 2

A 23-yr-old woman with end-stage renal disease caused by IgA nephropathy received a KT with 0% calculated PRA from her mother in August 2011. Three years later, her creatinine level increased to 2.24 mg/dL, and a biopsy of the allograft kidney revealed active T-cell-mediated rejection (TCMR). Four months later, the creatinine level increased to 6.17 mg/dL, and the allograft biopsy revealed TCMR with active ABMR, grade II with C4d negative, g1, and ptc3. Several class II HLA antibodies were identified by SABA. Both donor and recipient's HLA-DQ antigens were identified as HLA-DQ5 and DQ7. However, the HLA-DQ7 antibody reactions in serum were positive against two of the three Immucor DQ7 beads and three of five One Lambda beads. High-resolution *HLA-DQB1* and *DQA1* typing revealed donor *HLA-DQA1*06:01* that was different from patient *HLA-DQA1* gene.

The HLA antibody reactions were positive for *HLA-DQA1*04:01, DQA1*05:01, DQA1*05:03, DQA1*05:05,* and *DQA1*06:01*; these alleles share a confirmed epitope, $40GR_3$ (40G 41R 45V 47C 48L 50V 51L 52R 53Q 54F 55R, according to the HLA epitope registry). Therefore, the recipient antibody reaction was identified as *HLA-DQA1*06:01*-specific DSHA (Table 2).

HLA antibodies against DQ alpha antigens are observed in up to 79% of highly sensitized patients [5]. HLA-DQ antibodies show activity against both DQ alpha and beta proteins; however, their specific reactions against each type of protein cannot be easily differentiated. Our second case did not exhibit HLA antibodies prior to KT; however, HLA-DQ alpha protein-specific DSHA developed four years after transplantation, and the allograft biopsy revealed C4d negative ABMR.

Allele-specific DSHAs have been reported to have clinical significances [4]. However, only one of 67 crossmatches was positive for allele-specific DSHAs [6]. Therefore, the clinical significance of these DSHAs requires further investigation.

Our two cases showed biopsy-proven ABMR due to *HLA-DQB1* allele-specific DSHA and DQ alpha protein-specific DSHA. Although HLA-DQ typing is not included in routine HLA antigen-matching strategies, it must be performed to evaluate DSHA following KT. In addition, high-resolution *HLA-DQB1* and *DQA1* typing results should be considered when necessary for the interpretation of DSHAs in SABA.

Authors' Disclosures of Potential Conflicts of Interest

No conflicts of interest relevant to this article were reported.

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