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DP reactive antibody in a zero mismatch renal transplant pair

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Summary This study describes molecular basis for positive B-cell flow crossmatch in a zero mismatch (OMM) transplant pair. Our end-stage renal disease patient was B-cell flow crossmatch positive with a OMM deceased donor. DNA from the donor and patient were further typed by a high-resolution method. The patient's sera were tested for anti-HLA reactivity by single antigen bead using a Luminex platform. The patient and donor were found to be HLA identical except for a single DP allele MM (DPB1*0601). As there was no single antigen bead coated with DPB*0601, analysis of the amino acid residues of reactive and nonreactive DPB1 alleles was conducted. The results showed that all reactive alleles carried the amino acid D-E at residue 55 and 56 of DPB1. The MM allele DPB1*0601 also carries the DE 55-56 epitope. We conclude that positive B-cell flow crossmatch was likely the result of single MM in the DP locus.

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Introduction

The role of human leukocyte antigen (HLA)-DP mismatches on renal allograft survival has remained controversial. Recent evidence suggests that whereas HLA-DP mismatches may have little or no influence on the outcome of primary renal transplants, DP mismatches may play an important role in the outcome of retransplantations [1-3], possibly because DP antigens were discovered in the restimulation experiments of previously primed T cells [4] and therefore they may play a role in restimulation of secondary allogeneic proliferative and cytotoxic

immune response. At present, the standards of the United Network for Organ Sharing do not require HLA laboratories to perform either DQ typing or DP antibody identification. In addition, until recently, reagents necessary to identify anti-DP antibodies were not readily available. However the recent development of microparticle beads coated with DP antigens has made it possible to explore the role of DP in renal transplantation [1,2]. Although more than 100 DPB*1 alleles have been identified [5], the repertoire of bead-bound DP molecules remains limited.

In this retrospective case study analysis we hypothesized that the donor-specific B-cell positive crossmatch in a zero mismatch (OMM) donor-recipient pair resulted from donor-specific anti-DP antibodies.

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ABBREVIATIONS

HLA	human leukocyte antigen
MCS	mean channel shift
MM	mismatch
OMM	zero mismatch
SAB	single antigen bead

Subjects and methods

Several sera from a highly sensitized (both Flow Class I and II panel reactive antibody >90%) 32-year-old woman were crossmatched with lymphocytes isolated from a OMM deceased donor for her potential fourth renal transplant. The HLA antigens of her three previous transplants are shown in Table 1. The patient and donors' lymphocytes were serologically typed and found to be HLA identical at HLA A, B, Cw, DR, and DQ loci. The initial T- and B-cell screening crossmatches using a complement-dependent cytotoxicity technique were negative. The final T-cell flow crossmatches were negative; however B-cell flow crossmatches were positive using multiple sera (Table 2). The patient had no autoantibodies as evidenced by negative complement-dependent cytotoxicity and flow crossmatches using autologous T and B lymphocytes.

We hypothesized that although the patient was HLA A, B, C, DR, and DQ matched with the donor, she might be mismatched at the HLA DP antigen and her positive B-cell crossmatch might be caused by donor-specific anti-DP antibodies. The patient was not transplanted because at our center retransplantation across positive donor-specific B-cell crossmatch is considered to be contraindicated. The next day DNA samples from both the patient and donor were typed by a high-resolution molecular method for all HLA alleles. The patient and the donor were HLA identical (A*0201, 1101, B*0702, 5601, Cw*0102, 0702, DRB1*0101, 1501, DQB1*0501, 0601) except for one mismatch at a single DP allele in that the patient carried DPB1*0401, 0501 and the donor carried DPB1*0501, 0601. Serum samples from the patient were further analyzed for anti-DP antibodies by single antigen beads (SAB) (One Lambda, Canoga Park, CA).

Results

The antibody screening test with SAB showed broad sensitization against many allele groups of HLA-A and C and almost all allele groups of HLA-B, DR and DQ. Her sera did not react with the alleles of the following groups: HLA-A2, 3, 11, 19, B7, 42, 22, and the alleles of the KIR-ligand group 1 of HLA-C (Cw1, 3, 7, 8, 12, 14, 16) DR1, 2 DRw51 and DQ1. Interestingly her sera reacted with all alleles of DRB1*11 as well as the SAB containing DPB1*1701, 1001, 0301, 0201. Her sera did not react with the beads containing DPB1* 0101, 0401, 0501, 1301. As there were no SAB coated with the mis-

matched allele DPB1*0601, analysis of amino acid residue reactive and nonreactive DPB1 allele was carried out. The data showed that all reactive alleles carried the amino acid "D-E" at 55 and 56 of DPB1. DPB1*0601 also carries the "DE-55-56" epitope. DPB1*0401 allele does not contain the DE 55-56 epitope. Monoclonal antibody ILR1 reacts with this epitope as well as all alleles of DRB1*11 [6]. Several serum samples of the patient have shown a strong presence of DR11 antibody. On the basis of these data we hypothesized that DR11 antibody cross-reacted with the mismatched allele DPB1*0601.

Discussion

The classical HLA locus alleles (A, B, Cw, DRB, and DQB) expressed by our patient and the donor (A*0201, 1101, B*0702, 5601, Cw*0102, 0702, DRB1*0101, 1501, DQB1*0501, 0601) are common and well conserved and yet donor specific B-cell flow crossmatch was positive, suggesting that positivity caused by allelic variation was unlikely. It was hypothesized that positive B-cell donor-specific crossmatch was a result of sensitization of patient against DP antigens. It has been described by Flomenberg *et al.* [7] that donor-recipient pairs identical at all classical loci have 80% probability of being mismatched at DP loci.

Our data supported our hypothesis that the patient who was matched at all classical HLA loci with the donor was mismatched at the DP locus and consequently had developed antibodies against mismatched allele DPB1*0601; therefore donor-specific positive flow B-cell crossmatch was likely the result of a single antigen mismatch in the HLA DP locus.

This case study demonstrates that identity at HLA-A, -B, -C, -DR, -DQ loci between a transplant pair guarantees neither identity at the HLA DP locus nor negative crossmatch. Furthermore a highly immunized patient may have developed anti-DP antibody response, as detected by positive B-cell crossmatches between our patient and her OMM renal transplant donor. Our patient was mismatched only at DPB1*0601. Bodmer *et al.* [6] have shown that monoclonal antibody ILR1 is specific to the amino acid residues DE 55-56 of DPB1. The mismatched allele DPB1*0601 also carries the "DE-55-56" epitope. Among all DR alleles, the alleles of the DRB1*11 group are the only DR alleles that carry "D-E" at residues 57 and 58. Our patient's sera have expressed the strong presence of DR11 antibody (titer 1:64). We postulate that immunization by the more densely expressed DR11 molecule could have resulted in the patient's humoral sensitization against an interlocus DR-DP epitope. In fact it is hypothesized that the DPB1 molecule is shorter by two amino acids than the DRB1*11 molecules; but other than that, the surrounding sequences of the reactive DPB1 alleles and DRB1*11 alleles are identical [6].

Table 1 History of previous transplants

No	Tx date	Type 1	Graft loss	Donor HLA
1	7/86	DD	Chronic rejection	A2, 29, B35, B44 (DR, DQ not done)
2	2/88	LURD	Acute rejection	A1, 2, B7, 27, Cw2, DR1, w6 (w52), DQ1
3	1/94	DD	Chronic rejection	A2, B7, 50, Cw6, 7, DR3 (w52), 7 (w53), DQ2

DD, deceased donor; LURD, living unrelated donor; TX, transplant.

Table 2 Flow crossmatch results

Test reagents	T-cell channel	T-cell MCS (P/N)	B-cell channel	B-cell MCS (P/N)
Medium control	72		86	
Serum 1	74	2 (N)	127	41 (P)
Serum 2	73	1 (N)	135	49 (P)
Serum 3	71	0 (N)	116	30 (P)
Serum 4	77	5 (N)	149	63 (P)
Serum 5	70	0 (N)	124	38 (P)
Positive control (ALS)	172	100	206	120

N, negative, P, positive.

Interpretation: MCS (mean channel shift) ≥ 20 is considered positive for T cell and ≥ 30 is considered positive for B-cell flow crossmatches, respectively, using 256 channel resolution.

Patient's HLA = A2, 11, B7 (w6), 56 (w6), Cw 1,7, DR 1, 15 (w51), DQ5,6, DP 4, 5.

Donor's HLA = A2, 11, B7, (w6), 56 (w6), Cw 1,7, DR 1, 15 (w51), DQ 5,6, DP4, 6.

From a practical standpoint this case study raises several important questions. Can aggressive immunosuppressive therapy overcome rejection mediated by donor-specific anti-DP antibodies? Kamoun *et al.* reported a case [8] of a multiparous, highly sensitized recipient who received her third OMM kidney transplant across positive B-cell flow crossmatch because of anti-DP antibody. She received plasmapheresis, along with seven doses of thymoglobulin, OKT3, and four doses of Rituximab to overcome several bouts of acute antibody-mediated and cellular rejections. She remained dialysis free for 1 year with serum creatinine levels of approximately 2.1 mg/dl.

Their second case (personal communication with Dr. Kamoun) involved a human immunodeficiency virus (HIV)-positive renal recipient transplanted across OMM deceased donor kidney who also had donor-specific anti-DP antibody and positive B-cell crossmatch. He has undergone a number of rejection crises and has experienced many other complications, because he could not be treated aggressively with appropriate immunosuppressive therapy given his HIV positive status. As of this writing, his serum creatinine continues to increase, although he is not yet on dialysis. The allograft was lost because of rejection. These two case studies indicate that transplantation across positive B-cell crossmatch resulting from donor-specific DP antibody is possible if a patient can tolerate aggressive immunosuppressive therapy.

The United Network for Organ Sharing is in the process of developing a policy whereby a typing laboratory may be required to predict deceased donor-specific crossmatches with a high degree of accuracy before a donor kidney is shipped to a particular transplant center. To comply, a laboratory must identify individual HLA antibody in the sera of highly sensitized patients to determine acceptable versus unacceptable HLA MM for the patients. Acceptable MM defined on the basis of serologic identification of classical HLA (A, B, and DR) specificities may not be sufficient. To predict donor-specific crossmatches with a high degree of accuracy it may be necessary to identify both the HLA alleles of donor and patient and HLA antibodies in the patient's sera at allele levels by solid-phase assay.

We conclude that DPB1 typing of donors and highly sensitized patients, as well as detailed DP antibody screening, may be recommended for highly sensitized solid organ recipients.

Postscript

During the preparation of this manuscript and 15 months later, the patient described here received a 0 HLA A, B, DR MM kidney across a negative T- and B-cell flow crossmatch. Retrospective high-resolution molecular typing demonstrated that the patient was mismatched at both the DP loci (Donor DP B1*0601, 0402). Her historic serum was not used in the latest donor crossmatches. The sera used in the crossmatches had no anti-DP antibodies. She was discharged 1 week post-transplantation with serum creatinine 1.3 mg/dl. So far 3 months post transplantation she has not experienced any rejection episode.

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