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Donor-specific antibody to trans-encoded donor HLA-DQ heterodimer



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ABSTRACT

The majority of de novo donor specific HLA antibodies (DSAs) in transplant patients are directed to HLA-DQ antigens, which consist of a heterodimer of alpha and beta chains. Although a heterodimer can theoretically be cis- or trans-encoded, the sensitizing forms generally appear to be forms. DSA to DQ trans-heterodimer has never been reported. We reviewed 360 post-kidney transplant recipients (transplant: 2002–2013; follow-up: 5.6 ± 3.3 years). DQ DSA was detected in 46 of 57 patients who developed DSA. DSA specificity was consistent with donor mismatched DQ trans-heterodimers in three patients: DQ2.5 (DQB1*02, DQA1*05), DQ2.3 (DQB1*02, DQA1*03), and DQ4.3 (DQB1*04, DQA1*03). Two of them eventually lost grafts (2 and 5 years later) with allograft nephropathy. In conclusion, post-transplant patients may develop DSA to donor DQ trans-heterodimers. Further studies are warranted to determine the clinical significance of such DSAs.

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1. Introduction

Donor specific HLA antibody (DSA) is associated with risk of graft loss due to antibody-mediated rejection (AMR). DSA may develop any time after transplantation [1,2]. The majority of de novo DSA after kidney transplantation is directed to donor HLA-DQ antigens [3,4]. Although post-transplant DSA monitoring has been greatly improved with the recent use of solid phase single antigen techniques, post-transplant monitoring and detection of de novo DQ DSA can be challenging due to the complexity of HLA-DQ antigens [5].

Abbreviations: AMR, antibody-mediated rejection; CDC, complement-dependent cytotoxicity; DSA, donor-specific HLA antibodies; MFI, mean fluorescence intensity; SAB, single antigen bead; TCMR, T cell-mediated rejection.

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A HLA-DQ antigen is a heterodimer, comprising of one DQ alpha chain encoded by the *DQA1* gene and one DQ beta chain encoded by the *DQB1* gene. Genes, *DQA1*, *DQB1*, and *DRB1* are in strong linkage disequilibrium and are inherited en-bloc (*DRB1-DQA1-DQB1* haplotype) [6–11]. The possible combinations of heterodimers can be a cis- (a beta-chain and an alpha-chain from the same chromosome), or a trans- (a beta-chain from one chromosome and an alpha-chain from the other chromosome), thus theoretically up to four different DQ antigens may be expressed in an individual [12]. Since both alpha and beta DQ chains are polymorphic, a DQ antigen can be recognized as an allo-antigen if at least one of the chains is non-self (mismatched) [5]. Resulting DSA can be directed to a DQ alpha chain, a DQ beta chain, or both components of a DQ alpha and a DQ beta that consists of the cis- or trans-heterodimer.

Although a heterodimer can theoretically be cis- or trans-encoded, most DQ DSA in post-transplant patients are detected by antibody binding to DQ alpha-chain, DQ beta-chain, or DQ cis-heterodimers on test beads, explained as sensitization by

cis-heterodimer. So far, there are no published reports of de novo DSA against donor DQ trans-heterodimers. Therefore, sensitizing forms are generally considered as cis-heterodimers. It remains unknown if DQ trans-heterodimers can be expressed in donor allograft, can cause sensitization in recipients, and can be involved in the development of AMR. Therefore, we looked for de novo HLA antibody with specificity consistent with donor DQ trans-heterodimers in post-kidney transplant patients and found in three patients. The three patients we report in this case study may represent potential evidence of sensitization by mismatched DQ trans-heterodimer.

2. Materials and methods

Retrospective review of DSA and clinical course of patients who received ABO and lymphocyte crossmatch compatible kidney transplantation between 2002 and 2013 was performed. Of all the patients who were tested for post-transplant DSA, excluded were patients with less than one year follow-up and patients who received additional non-renal transplantations. Testing and use of patient data were approved by the Penn State University College of Medicine Institutional Review Board.

Patients received induction treatment with anti-thymocyte globulin (adult) or alemtuzumab (pediatrics) accompanied by methylprednisolone pre-medication, followed by maintenance treatment with tacrolimus and mycophenolate mofetil. Prednisone with tapering was added for adult patients. Patients were switched to cyclosporin A for maintenance, if they developed complications related to tacrolimus.

HLA-A, B, and C were typed either by a serological or a molecular method. DR (*DRB1*/3/4/5), DQ (*DQB1*/*DQA1*), and DP (*DPB1*/*DPA1*) were typed by molecular method. *DQB1*-*DQA1*-*DRB1* haplotypes were assigned by gene association [6,13]. *DQA1* and *DPB1*/*DPA1* typings were instituted since 2010 and 2013, respectively. When donor *DQA1* type was not available, *DQA1* was re-typed from frozen cells or predicted by gene association [6,13].

HLA antibody was tested by Luminex microbead array (Labscreen PRA/Single Antigen bead (SAB), One Lambda) on pre-treated serum with dithiothreitol (DTT) [14] or EDTA [15]. DSA was tested at post-transplant Day 0 (the day of transplant), Day 7, monthly until Month 6, every two months until Year 1, every 6 months until Year 3, annually thereafter, and any time clinically indicated. DSA was assigned as positive when detected at >500 mean fluorescence intensity (MFI), which is lower than 1000 MFI threshold generally used for pre-transplant patients. DSA strength was considered weak (equivalent to negative flow crossmatch), moderate (equivalent to positive flow crossmatch but negative CDC crossmatch), or strong (equivalent to positive CDC crossmatch) [16]. In this study, DSA was considered de novo when the first detection was after at least one negative DSA result and >10 days after transplantation. Complement fixing ability of DSA was evaluated by C1q assay (One Lambda). T and B lymphocyte crossmatch were performed by flow cytometry method [16] (from mid-2008 to present) or AHG-enhanced CDC method (from 2002 to mid-2008).

3. Results

3.1. De novo DSA (Table 1)

Total of 360 post-transplant kidney recipients were reviewed. The mean age at the time of transplant was 43.9 years (range: 2–78 years) and the mean follow-up period was 5.6 ± 3.3 years. De novo DSA was detected in 57 of 360 patients (15.8%, Table 1). DQ DSA was detected in 46 patients. In three patients, DQ DSA was

Table 1
Specificity of donor specific antibody (DSA).

De novo DSA specificity (IgG)	Patient, N (%)
A	13 (22.8%)
B	6 (10.5%)
C	0 (0%)
DR (<i>DRB1</i>)	6 (10.5%)
DR (<i>DRB3</i> /4/5)	5 (8.8%)
DQ	
α chain, β chain, or cis-heterodimer	43 (75.4%)
Trans-heterodimer	3 (5.3%)
Total	*57 (100%)

* Of the 57 patients, 13 patients had more than one DSA (total of 76 DSAs).

detected as reactivity to the trans-combinations of donor alpha and beta chain components, thus consistent to predicted DQ trans-heterodimer. In the remaining 43 patients with DQ DSA, specificity was detected as donor DQ alpha-chain ($N = 10$), DQ beta-chain ($N = 5$), DQ alpha–beta cis-heterodimer ($N = 12$), and undetermined due to the insufficient resolution of the test beads ($N = 16$).

3.2. Patients with de novo DSA to predicted DQ trans-heterodimer (Table 1, Fig 1)

Patient 1 was a Caucasian female with a history of end-stage renal disease due to type I diabetes. She received a kidney transplant from a living unrelated Caucasian donor with negative CDC crossmatch when she was 50 years-old (2005). Patient's DQ antigen was DQ8 (*DQA1**03) (Fig 1A). Donor class II antigens were DR11 DR7 DR52 DQ2 DQ7, thus the most likely DQ haplotype is *DQB1**02:01P-*DQA1**02:01 (*DQ2.2*) and *DQB1**03:01-*DQA1**05:01 (*DQ7.5*) (Fig. 1B). Serum creatinine was 1.2 mg/dL and HLA antibodies were negative for the first 3.8 years. De novo HLA antibody was first detected in the post-transplant year 4 with reactivity to a test bead with *DQB1**02-*DQA1**05 (*DQ2.5*), consistent with presence of DSA to a DQ trans-heterodimer. No additional reactivity was detected to the donor's remaining DQ antigens (beta-chain alone: DQ2, DQ7, alpha-chain alone: *DQA1**02, *DQA1**05, and cis-heterodimers: DQ7.5, DQ2.2). Biopsy showed focal positive C4d staining with no evidence of T cell-mediated rejection (TCMR). Initially, creatinine was stable and repeat biopsies were negative for C4d staining and TCMR, thus patient was not treated for AMR. However, DQ2.5 DSA gradually increased to strong level and the reactivity expanded to include DQ7.5 (the donor cis-heterodimer) and few non-DSAs. The DSA to DQ2.5 remained the highest and the only one found to be complement fixing. Creatinine also gradually increased and graft function was lost at the post-transplant year 9 (5 years after the emergence of anti-DQ2.5) with a biopsy finding of severe interstitial fibrosis/tubular atrophy, consistent with progressive allograft nephropathy.

Patient 2 was a Caucasian male with a history of Alport syndrome and end-stage renal disease due to hereditary nephritis. He received a kidney transplant from a living unrelated Caucasian donor with negative CDC crossmatch when he was 21 years-old (2006). Patient's DQ antigens were DQ8 DQ6 (*DQA1**01, 03) (Fig 1A). Donor class II antigens were DR4 DR7 DR53 DQ2 DQ8, thus the most likely DQ haplotype is *DQB1**02:01P-*DQA1**02:01 (*DQ2.2*) and *DQB1**03:02-*DQA1**03:01 (*DQ8.3*) [6]. Baseline creatinine was 1.6 mg/dL and HLA antibody was negative for 3 years. In the post-transplant year 5, creatinine increased to 2.3 mg/dL. De novo HLA antibody was detected and was most reactive to a test bead with *DQB1**02-*DQA1**03 (*DQ2.3*), consistent with presence of DSA to a DQ trans-heterodimer. The reactivity to the remaining donor's DQ antigens (beta-chain alone: DQ2, DQ8,

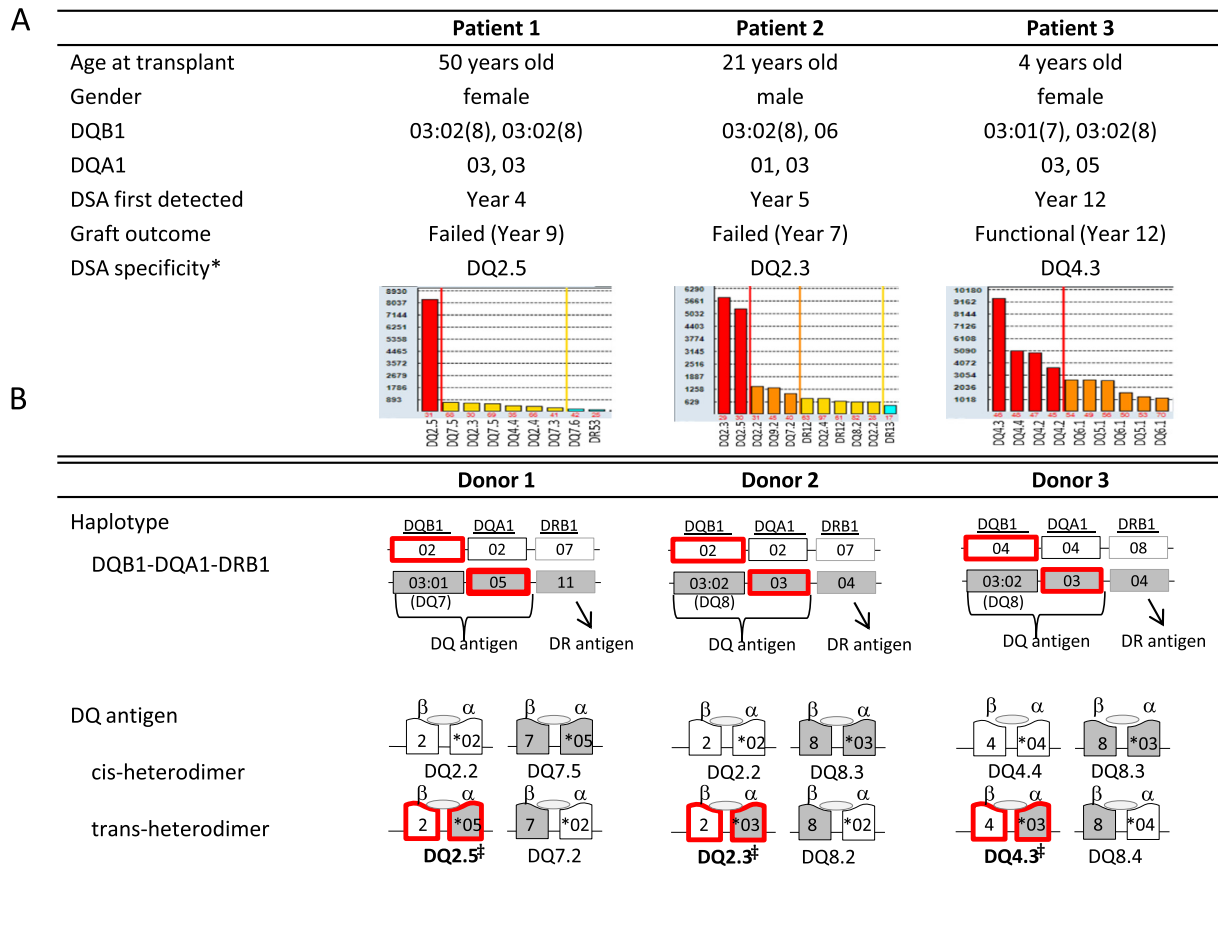


Fig. 1. (A) Patient's demographic data, HLA-DQB1/DQA1, donor specific HLA antibody (DSA) and graft outcome are shown. ^{*}DSA specificity was assigned by the HLA antigen on test beads that demonstrated the highest reactivity; (B) Donor *DQB1-DQA1-DRB1* haplotypes and heterodimer configuration (cis- and trans-) of DQ antigen. [‡]DQ trans-heterodimer consistent with DSA.

alpha-chain alone: DQA1*02, DQA1*03, cis-heterodimers: DQ2.2, DQ8.3) were either negative or weak. Complement fixing ability was not detected in any of the HLA specificities. Biopsy showed diffuse C4d staining and borderline cellular rejection. Patient was diagnosed with mixed rejection (AMR and TCMR). After the treatment (plasmapheresis, intravenous immunoglobulin, rituximab, and steroid), creatinine decreased to 1.9 mg/dL and biopsy verified the resolution of TCMR and negative C4d staining. However anti-DQ2.3 antibody persisted at a weak level and the graft was lost in the post-transplant year 7 (two years after the emergence of anti-DQ2.3) with biopsy findings of severe interstitial fibrosis and tubular atrophy.

Patient 3 was a Hispanic female with a history of end-stage kidney disease due to familial focal segmental glomerulosclerosis. She received a kidney from her father with negative CDC cross-match when she was 4 years-old (2002). Patient's DQ antigens were DQ7 DQ8 (DQA1*03, 05) (Fig 1A). Donor class II antigens were DR4 DR8 DR53 DQ4 DQ8, thus the most likely DQ haplotype is *DQB1*04:02-DQA1*04:01* (DQ4.4) and *DQB1*03:02-DQA1*03:01* (DQ8.3) (Fig. 1B). For 8 years, creatinine was 0.9 mg/dL and HLA antibody was negative. De novo HLA antibody was found to be positive in the post-transplant year 12 and was most reactive to a bead with DQB1*04-DQA1*03 (DQ4.3), consistent with presence of DSA to a DQ trans-heterodimer. The reactivity to the remaining donor's DQ antigens (beta-chain alone: DQ4, DQ8; alpha-chain alone: DQA1*02, DQA1*04; cis-heterodimers: DQ4.4, DQ8.3) were negative or weak. None of the specificities were detected as

complement fixing. Increased creatinine of 1.4 mg/dL and TCMR (Banff; grade Ia) with negative C4d staining were also detected (biopsy). Graft function improved with steroid treatment and has remained stable with persistent moderate anti-DQ4.3.

4. Discussion

HLA-DQ antigen mismatch between donor and recipient is a major risk factor for developing de novo DSA [17,18]. Many post-transplant patients develop de novo DSA to donor DQ antigens, which often precede chronic/indolent AMR and subsequent graft loss [1,2]. In our cohort, of the 57 DSA positive patients, DQ DSA was detected in 46 patients including the three patients with de novo HLA antibody to predicted DQ trans-heterodimers. Thus, DSA recognized with specificity to DQ trans-heterodimer is relatively infrequent (3/46 DQ DSA patients, 6.5%).

Despite the theoretical presence of DQ trans-heterodimers in tissue, expression has never been demonstrated in donor allograft. While the question remains if it can be actually expressed and sensitize transplant recipients, the expression of trans-heterodimers is supported by disease association between certain HLA haplotypes and celiac disease [12]. Increased risk of celiac disease is known in individuals with the HLA-DQ2.5 or DQ8 which mediate gluten epitopes presentation to intestinal T-cells [19]. Interestingly, individuals who have *DQB1*02* and *DQA1*05* in trans-encoded configuration are also at increased risk of celiac disease (for example,

haplotypes with *DQB1*02-DQA1*02* and *DQB1*03:01-DQA1*05*) [12]). Trans-heterodimer DQ2.5 was one of the few stable DQ trans-heterodimers of which expression has been demonstrated experimentally in a cell culture system [20]. Not all DQ trans-heterodimers are universally stable, which may explain why DSA to DQ trans-heterodimers are less frequent than DQ cis-heterodimers.

In addition to the strongest reactivity to the predicted donor DQ trans-heterodimer antigen, patient's serum also show weaker reactivities to donor DQ cis-heterodimer antigens and non-donor DQ antigens (Fig 1). These reactivities can be explained by a DSA and its crossreactivity to the shared epitopes of HLA-DQ alpha or beta chains. Rather, presence of multiple antibodies is less likely because the reactivities appeared simultaneously in these patients. We concluded that three patients developed de novo HLA antibody directed to a predicted trans-heterodimer, which is likely DSA because: (1) all three patients were not sensitized to donor antigens at the time of transplant (negative lymphocyte crossmatch) and had been tested negative for HLA antibody prior to the developing of HLA antibody, (2) there had been no other sensitization events other than presence of allograft that coincided with developing HLA antibody, and (3) positive C4d staining was documented in biopsy in two patients.

One limitation of this study is the lack of donor *DQA1* typing. Importance of donor *DQA1* typing in solid organ transplantation has only been established recently, thus most donors in the United States were not typed for *DQA1* until recently. Although prediction of *DQA1* genotype based on *DRB1-DQB1-DQA1* haplotype association is not perfect, we believe that the predicted *DQA1* typing is correct in these three cases because the predicted *DRB1-DQB1-DQA1* haplotypes show strong linkage regardless of ethnicity [6–11].

After developing de novo DSA to the donor's mismatched DQ trans-heterodimers, two of the three patients eventually lost grafts. At this time, we do not have definitive evidence that graft loss was mediated by the DQ trans-heterodimer DSAs. Diagnosis of AMR was not established in Patient 1 who had stable creatinine and negative C4d staining by repeated biopsy. Similarly, in Patient 2, AMR was no longer suggested after the treatment since resolution of TCMR and C4d staining were both verified by biopsy. TCMR can be ruled out as a cause of graft loss in Patient 2, because creatinine recovered and has been stable after the treatment and resolution of TCMR. On the other hand, diagnosis of AMR, especially "C4d-negative AMR" is difficult [21]. AMR by de novo DSA is often chronic/indolent [1], negative for C4d staining [22], can overlap with TCMR [23], and a major cause of late graft loss [21].

In conclusion, DSA can be detected with specificity to DQ trans-heterodimers, which may represent potential evidence of sensitization by mismatched DQ trans-heterodimer. Further studies are warranted to evaluate if DQ trans-heterodimer DSA is associated with increased risk of AMR and graft loss.

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