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Preformed Donor HLA-DP-Specific Antibodies Mediate Acute and Chronic Antibody-Mediated Rejection Following Renal Transplantation

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Abstract

Donor-specific HLA alloantibodies may cause acute and chronic antibody-mediated rejection (AMR) and significantly compromise allograft survival. The clinical relevance of antibodies directed against some HLA class II antigens, particularly HLA-DP, is less clear with conflicting reports on their pathogenicity. We report two patients with high levels of pretransplant donor-specific HLA-DP antibodies who subsequently developed recurrent acute AMR and graft failure. In both cases, there were no other donor-specific HLA alloantibodies, suggesting that the HLA-DP-specific antibodies may be directly pathogenic.

Keywords

Antibody-mediated rejection; HLA alloantibodies; renal transplantation

Background

Donor-specific HLA alloantibodies can cause hyperacute, acute and chronic antibody-mediated rejection (AMR) (1). However, not all HLA alloantibodies are considered pathogenic. Low expression of HLA-DP in renal endothelial cells (2) has led to the notion that DP-specific alloantibodies are of minimal significance, a concept supported by the observation that in a single study, HLA-DP antibody-positive renal transplant recipients did not have impaired allograft survival (3). However, HLA-DP mismatch is associated with reduced graft survival in retransplants (4,5), particularly in patients with high panel reactivity of lymphocytotoxic antibodies (4). More recently, a small number of reports have

suggested that donor-specific HLA-DP antibodies can directly mediate allograft damage (6–9).

We report two renal transplant recipients with pretransplant donor-specific HLA-DP antibodies who experienced recurrent AMR and allograft loss. In both cases, there were no other donor-specific HLA antibodies, suggesting that HLA-DP antibodies were directly pathogenic.

Case 1

A 50-year-old Caucasian male with end-stage renal disease (ESRD) secondary to chronic pyelonephritis, and two previous failed deceased donor kidney transplants, received a donation after brain death (DBD) donor kidney. Pretransplant antibody screening was performed using a dithiothreitol (DTT)-modified complement-dependent cytotoxicity (CDC) assay, as described previously (10). Antibody characterization was undertaken using HLA class I and class II single-antigen HLA-specific antibody detection beads (LABScreen®; One Lambda Inc., Canoga Park, CA, USA). The patient was found to be highly sensitized, with strong IgG antibodies to multiple HLA class I and class II specificities including HLA-DPB1*01 (mean fluorescence intensity (MFI): 9280). This, his third transplant, carried no mismatches at HLA-A, -B, -DR or -DQ loci and was mismatched only for HLA-C*15 and HLA-DPB1*01 (Table 1a). As expected, the pretransplant CDC and flow cytometric crossmatch (FCXM) was T cell negative and B cell positive. An autologous T- and B cell FCXM was negative, indicating that the positive B cell crossmatch was likely caused by HLA-DPB1*01 antibody. Screening for antibodies to MHC-Class I-related chain A (MICA) was negative.

The patient received an anti-CD25 monoclonal antibody at induction, followed by tacrolimus, mycophenolate mofetil (MMF) and prednisolone. The allograft did not function immediately, and on day 3, a biopsy demonstrated possible calcineurin inhibitor toxicity and acute cellular rejection (Banff IIA), for which he was treated with intravenous methylprednisolone (IVMP). Donor-specific HLA-DPB1*01 antibody levels on day 9 posttransplant were lower than pretransplant levels (MFI: 5666).

Allograft function slowly improved such that his serum creatinine was 3 mg/dL 4 weeks following transplantation. Given the suboptimal graft function, he underwent a repeat biopsy, which showed glomerular mesangiolysis, fibrinoid necrosis and C4d staining in peritubular capillaries (PTC), consistent with AMR. He was treated with IVMP and four sessions of double filtration plasmapheresis (DFPP). Two weeks later, donor-specific HLA-DPB1*01 antibodies were reduced (MFI: 425) but a further biopsy demonstrated persistent AMR for which he was commenced on anti-thymocyte globulin (ATG). After four doses of ATG, the patient developed pancytopenia and septicemia, necessitating intensive care admission. Unfortunately, the patient subsequently developed further infectious complications over the next 18 months, including recurrent urosepsis and a cavitating lung lesion (secondary to mucormycosis), necessitating a right lower lobectomy and prolonged antifungal treatment. His graft function continued to deteriorate such that he became dialysis-dependent at 20 months posttransplant and underwent a graft nephrectomy.

Histological examination demonstrated chronic AMR. No donor-specific antibody (DSA) other than the DP antibody was detectable throughout the posttransplant period.

Case 2

A 47-year-old Caucasian male with ESRD secondary to urethral valve-associated obstructive uropathy and a previous DBD donor kidney transplant received a living, unrelated transplant from his wife (1-2-1 HLA-A, -B, -DR mismatched graft, with an additional single mismatch at the DP locus (Table 1b)). HLA-DP typing was performed by sequence-based typing which identified his wife's HLA-DP type as HLA-DPB*04:01 and 03:01/05:02 (the polymorphism which distinguishes HLA-DPB1*03:01 and 05:02 lies outside the sequenced region; therefore, these two alleles cannot be distinguished by the method used at our center). The patient did not have detectable donor-specific HLA-A, -B, -DR or -DQ antibodies, nor MICA antibodies, but single antigen bead (SAB) testing demonstrated high-level antibodies to both HLA-DPB1*03 and HLA-DPB1*05. The T cell CDC crossmatch was negative but the B cell CDC and FCXM were positive. In view of the CDC-positive crossmatch and the high-level donor-specific DP antibody, the patient underwent antibody reduction therapy prior to transplantation, receiving rituximab at day -28, tacrolimus, MMF and prednisolone at day -14, alemtuzumab at day 0 and 7 and DFPP both prior to (seven sessions) and following (nine sessions) transplantation. The pre-DFPP donor HLA-DPB1*03- and HLA-DPB1*05-specific antibody levels were saturating using undiluted serum, and giving a MFI of 13 216 and 12 453, respectively, using 1:100 serum dilution. DFPP resulted in a reduction in donor-specific HLA-DP antibody levels (MFI: 5894 and 6892 at 1:100 dilution). Following transplantation, there was immediate graft function and the patient was discharged on day 9 with a serum creatinine of 1.9 mg/dL. On day 11 posttransplant, the serum creatinine increased to 3.7 mg/dL and a biopsy demonstrated neutrophils within the lumen of PTC and C4d staining in 100% of PTC, diagnostic of AMR (Figure 1a). Consistent with this, donor-specific HLA-DP antibodies were increased compared with the immediate pretransplant levels. The patient was treated with five sessions of DFPP and ATG, following which there was an improvement in renal function (creatinine 2 mg/dL).

Twelve months posttransplant, renal function deteriorated and a biopsy demonstrated acute-on-chronic AMR (with C4d staining in 95% of PTC) and chronic vascular changes (Figure 1b). He was treated with IVMP, five sessions of DFPP and a 2-week course of ATG. A repeat biopsy showed ongoing low-grade AMR and mild acute cellular rejection (Banff 1B). Despite further DFPP and intravenous immunoglobulin, his graft function continued to decline and a biopsy demonstrated persistent acute on chronic AMR. Given these chronic histological changes and the presence of low-grade cytomegalovirus (CMV) and JC viraemia, the risk of infectious complications was felt to outweigh any potential benefit of further immunosuppression. He was therefore commenced on hemodialysis, and subsequently underwent a graft nephrectomy. Of note, no DSA other than the HLA-DP antibody was detectable during the 12 months posttransplant.

Given our experience with these two patients, we sought to determine the prevalence and significance of pretransplant HLA-DP-specific antibodies in renal transplant recipients at

our center. We retrospectively gathered data on all kidney transplant recipients ($n = 550$) transplanted at Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK, between January 2007 and April 2011 (deceased and living donors). This included case 2 (described above) but not case 1, who was transplanted prior to the time period assessed. Data collected included the pretransplant HLA-specific antibody profile (as defined using SAB, MFI >1500 , which is standard practice at our center). Donor and recipient HLADP genotyping was performed retrospectively in the patients in whom pretransplant HLA-DP-specific antibodies were identified.

Pretransplant HLA-DP-specific antibodies were detectable in 29 of the 550 patients (5.3% in line with previous studies (3, 11, 12)). Nineteen of these patients (65.5%) with pretransplant HLA-DP-specific antibodies had received a previous transplant.

Since DSA with a MFI lower than that used routinely at our center (MFI < 1500) might be pathologically significant, we used a more rigorous cutoff (MFI > 500) to identify six patients with isolated donor-specific DP antibodies, and no other DSA. In this subset, the frequency of AMR was 50% compared with 6.6% in patients with isolated nondonor-specific DP antibodies (Figure 2, $p = 0.02$ on multivariate analysis). However, these data are limited by the nature of the study, which was retrospective and had only a small number of subjects with isolated DP antibodies on which multivariate analysis could be performed. Of note, three patients with isolated HLA-DP antibodies had a negative CDC crossmatch, but subsequently went on to develop AMR, indicating the insensitivity of this method of screening for DSA.

Discussion

HLA-DP is a classical MHC class II molecule composed of polymorphic α and β chains (13). The majority of DPB1 alleles differ at six hypervariable regions in exon 2 of the β 1 domain at residues 8–11, 32–36, 55–57, 65–69, 76 and 84–87 (designated A–F). Combinations of amino acids in the six hypervariable regions characterize the different HLA-DP alleles (14). Although HLA-DP-specific antibodies are less common than antibodies to HLA-DR and -DQ (occurring in 5–14% of transplant recipients [3,11,15]), the frequency of HLA-DP antibodies increases in patients previously transplanted, occurring in up to 45% of subjects (15). In keeping with this, we observed DP antibodies in 5.3% of the patients transplanted at our center, and two-thirds of these had undergone a previous transplant.

HLA-DP is constitutively expressed in renal endothelial cells, but at low levels (2). Therefore, donor-specific HLA-DP antibodies have been perceived as relatively benign in renal transplant recipients. This view is supported by the observation that in a study of 505 patients, the presence of IgG HLA-DP-specific antibodies correlated weakly with a positive CDC crossmatch and HLA-DP antibody positive recipients did not have reduced allograft survival (3). However, although DP mismatch has no effect on graft survival in first transplants (4, 16), studies have demonstrated a significant effect in retransplants (4, 5). In a large study of 1300 patients undergoing repeat transplant, Mytilineos et al. observed a 1-year graft survival of 83% in patients with no HLA-DP mismatches, 76% in those with one HLA-

DP mismatch and 73% in patients with two HLA-DP mismatches (4). The deleterious influence of HLA-DP mismatch in this study was particularly strong in patients with high panel reactivity of preformed lymphocytotoxic antibodies, raising the possibility that donor-specific HLA-DP antibodies may have pathogenic effects. Furthermore, there are data suggesting that mismatch of specific immunogenic epitopes of HLA-DPB1 are of particular importance in determining transplant outcome (5).

The two cases we have reported strongly suggest that HLA-DP antibodies may be pathogenic, as neither patient had any other detectable donor-specific HLA or MICA antibodies. Of particular note is patient 2 who, despite a substantial antibody reduction protocol, experienced recurrent AMR culminating in allograft loss. Nelson et al. described two similar cases in which antibody reduction therapy in patients with isolated donor-specific HLA-DP antibody did not prevent AMR (6).

In summary, we have described two patients who developed recurrent AMR and allograft loss due to donor-specific HLA-DP antibodies. Therefore, sensitized patients with donor-specific HLA-DP antibodies should be carefully monitored posttransplant, with a low threshold for allograft biopsy and consideration given to pretransplant antibody reduction therapy in those with a persistently high antibody titer.

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Abbreviations

Ai	antibody incompatible
AMR	antibody-mediated rejection
ATG	anti-thymocyte globulin
CDC	complement-dependent cytotoxicity
CMV	cytomegalovirus
DBD	donation after brain death
DFPP	double filtration plasmapheresis
DSA	donor-specific antibody
DTT	dithiothreitol
ESRD	end-stage renal disease
FCXM	flow cytometric crossmatch
IVMP	intravenous methylprednisolone
MFI	mean fluorescence intensity
MICA	MHC-Class I-related chain A

MMF	mycophenolate mofetil
PRA	panel reactive antibody
PTC	peritubular capillaries
SAB	single antigen bead

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Figure 1.

(A) Renal allograft biopsy demonstrating acute AMR with 100% C4d positivity in PTC (arrow)

(B) Severe fibrous intimal thickening and lymphocytic endothelialitis of an interlobular artery diagnostic of chronic AMR with transplant vasculopathy. (Both images: H+E $\times 400$, kindly supplied by Dr. Meryl Griffiths, Consultant Histopathologist, Cambridge University Hospitals NHS Foundation Trust, Addenbrooke's Hospital, Cambridge, UK.)

HLA-DP Antibodies and Outcome Posttransplant

Figure 2.

Kaplan–Meier plot showing AMR-free survival for patients with isolated donor-specific DP (dsDP; n = 6) or isolated nondonor-specific DP (non-dsDP; n = 15)

Isolated dsDP was associated with significantly reduced AMR-free survival ($p = 0.02$ on multivariate analysis). No other variables were significant, including delayed graft function, donor type (living vs. deceased), antibody reduction protocol and HLA mismatch.

Maintenance immunosuppression was comparable between groups.

Table 1

(a) HLA typing of third donor and of recipient (case 1) and (b) HLA typing of second donor and of recipient (case 2)

a. Case1	HLA-A	HLA-B	HLA-Bw	HLA-Cw	HLA-DR	HLA-DRB1	HLA-DR51/52/53	HLA-DQB1	HLA-DPB1
Donor 3	1, 24(9)	8, 62(15)	6	7, 15	17(3)	*03:01	52	*02:01	*01:01, 02:01:02
Recipient	1, 24(9)	8, 62(15)	6	7, 9(3)	17(3), 4	*03:01, 04	52, 53	*02:01, 03:02	*04:01
b. Case2	HLA-A	HLA-B	HLA-Bw	HLA-Cw	HLA-DR	HLA-DRB1	HLA-DRB3-5	HLA-DQB1	HLA-DPB1
Donor 2	1, 24(9)	39(16), 72(70)	6	12	16(2), 7	*16, 07	51, 53	*05:02, 02:01	*04:01, 03:01/05:02
Recipient	1, 68(28)	8, 44(12)	4, 6	4, 7	7, 17(3)	*03:01, 07	52, 53	*02:01	*02:01, 04:01