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Pre-transplant donor specific anti-HLA antibody is associated with antibody-mediated rejection, progressive graft dysfunction and patient death ☆

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ABSTRACT

Background: The long term effect of donor specific antibodies (DSA) detected by Luminex Single Antigen Bead (SAB) assay in the absence of a positive complement-dependant cytotoxicity (CDC) crossmatch is unclear.
DSA at the time of transplant were determined retrospectively in 258 renal transplant recipients from 2003 to 2007 and their relationship with rejection and graft function prospectively evaluated.
After a median of 5.6 years follow-up 9% of patients had antibody mediated rejection (AMR) (DSA 11/37 (30%), DSA-Neg 13/221 (6%), HR 6.6, $p < 0.001$). Patients with anti-HLA class II (HR 6.1) or both class I + II (HR 10.1) DSA had the greatest risk for AMR. The Mean Fluorescent Intensity (MFI) of the DSA was significantly higher in patients with AMR than those with no rejection ($p = 0.006$). Moreover, the strength of the antibody was shown to be important, with the risk of AMR significantly greater in those with DSA >8000 MFI than those with DSA <8000 MFI (HR 23, $p < 0.001$).
eGFR progressively declined in patients with DSA but was stable in those without DSA (35.7 ± 20.4 mls/min vs 48.5 ± 22.7) and composite patient and graft survival was significantly worse in those with class II (HR 2.9) or both class I + II (HR 3.7) but not class I DSA. Class II DSA alone, or in combination with class I DSA had the strongest association with graft loss and patient death.
Patients with DSA not only have increased rates of acute AMR, but also chronic graft dysfunction, graft loss and death. Antibody burden quantified by SAB assay may identify patients at highest immunological risk and therefore influence patient management and improve long-term patient outcome.

1. Introduction

The use of the CDC T cell crossmatch for the detection of preformed anti-HLA class I antibody to eliminate hyperacute rejection has allowed renal transplantation to become the preferred method of management of end stage renal disease (ESRD) in eligible patients. Advances in immunosuppressive therapies have also reduced acute cellular rejection rates to very low levels [1]. However, despite these advances, improving long-term graft survival remains problematic [2]. Increasingly the role of antibody mediated graft injury, manifested either as acute humoral rejection or chronic AMR (transplant glomerulopathy), has been recognised as an important, potentially modifiable cause of graft attrition [3–6].

The introduction of sensitive and specific solid phase antibody detection assays allow detection of lower levels of antibody than those associated with a positive CDC T- or B-cell crossmatch. These assays can accurately determine the strength and specificity of antibodies, thereby improving the ability to examine their role in graft rejection. We, and others, have previously shown that the presence of anti-HLA antibodies detected after transplant is associated with a significant risk of subsequent graft loss [4,7–9] though it was not known whether these antibodies were present at the time of transplant or arose de novo. However, there is contradictory data on the significance of antibodies detected only by solid phase assay in the presence of a negative T-cell CDC crossmatch at the time of transplant.

Abbreviations: AMR, Antibody-mediated Rejection; CDC, Complement Dependant Cytotoxicity; cPRA, Calculated Panel Reactive Antibody; DSA, Donor Specific Antibodies; HB-MFI, Highest bead MFI; DGF, Delayed Graft Function; BPAR, Biopsy Proven Acute Rejection; MFI, Mean Fluorescence Intensity; SAB, Single Antigen Bead.

☆ Conflict of interest: The authors have declared that no conflict of interest exists.

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Whilst some recent studies have shown that DSA at the time of renal transplant are associated with increased risk of acute AMR [10–12] when the crossmatch is positive and others have shown that even with a negative crossmatch [9,12–15], pre-transplant DSA are associated with early AMR and rejection, there is little information on the influence of pre-transplant DSA on long term renal function.

Current literature is unclear in terms of the importance of class I versus class II versus strength of DSA with respect to AMR and graft loss, and these differences may be related to differences in transplant management and immunosuppressive protocols. We initially set out to determine how our data sit with respect to other publications in terms of AMR and graft loss so that graft function, as measured by eGFR, could be viewed in the context of the rest of our data.

Results presented here validate the association between pre-transplant DSA and AMR. We show that, like some others, classes I and II DSA are more important than either class I or II alone for AMR. However, we further propose that the class of DSA may be unimportant and the effects of DSA on graft rejection and outcome can be explained by antibody strength. These findings may help to explain the differing importance of classes I and II antibody between centres. We also show that DSA is associated with non-AMR though not as strongly as with AMR

2. Subjects and methods

2.1. Patients

We studied 267 patients with a negative T cell CDC crossmatch who underwent 270 consecutive renal transplants between June 2003 and October 2007. We excluded one patient with a simultaneous liver–kidney transplant, and those patients who died (n = 3) or lost their graft within the first 30 days of transplantation (n = 8; 3 of whom were subsequently re-transplanted within the period of observation), leaving 258 patients and allografts. Of the 258 patients, 173 were also B cell crossmatched. 246 patients received a kidney transplant in Perth, Western Australia and 12 patients received simultaneous kidney–pancreas transplants at Westmead Hospital in New South Wales. Patients were managed with varying immunosuppressive regimes but all patients received a Calcineurin inhibitor (CNI) (Tacrolimus or Cyclosporine) at the time of transplantation in combination with mycophenolate mofetil or mycophenolate sodium and corticosteroids. Consistent with Australian practice, the IL2R Antibody Basiliximab was commonly used for induction but the use of anti T-cell preparations for induction was rare (Table 1). The need for biopsy, diagnosis and management of rejection, medication adjustments was determined by the caring clinician and was not protocol driven.

2.2. Prospective testing

The Department of Clinical Immunology (DCI), Royal Perth Hospital performed all T and B-cell CDC crossmatching against WA donors. For organs donated from other Australian states the crossmatch was performed at the state of donor origin. Pre-transplant HLA typing and HLA antibody testing of the kidney recipients was performed by DCI. All deceased donors were typed for HLA-A, -B, -DR, -DR51, -DR52, -DR53 and -DQ by serology using commercial monoclonal antibody trays (OneLambda Inc). Living donors and all potential recipients were typed for HLA-A, -B, -DRB1 by DNA sequencing based typing (In-house) [16].

Donor and recipient HLA-A, -B and -DR matching, patient sensitisation (PRA) and waiting time were considered in recipient allocation. Antibodies directed against HLA-A and -B were determined by CDC crossmatch or Luminex PRA bead (OneLambda Inc). The presence of class I DSA in current or historical sera and previous donor class I or class II HLA mismatches (HLA-A, -B and -DR only) were exclusions for organ allocation. A negative pre-transplant T cell CDC crossmatch using current (within 3 months prior to the transplant) and historical sera was mandatory for transplantation. B cell crossmatching was performed for 67% of the patients, however a positive B cell crossmatch was not considered an absolute contraindication to transplantation.

2.3. Retrospective testing

For this study, sera collected at the time of transplant were screened retrospectively for anti-HLA class I and class II antibodies using the Luminex Mixed Screen assay and those with a positive screen were characterised for HLA class I and/or class II antibody specificity using Single Antigen beads (LABScreen Single Antigen beads, OneLambda Inc). The assays were performed in accordance with the manufacturer's protocol. Antibodies were considered to be positive if the MFI for a particular bead was greater than 500. HLA antibodies with an MFI > 500 directed against a donor HLA antigen (HLA-A, -B, -C, -DRB1, -DRB3,4,5, -DQB1, or -DPB1) were considered to be DSA.

Table 1
Demographic and clinical features at time of transplant according to entry antibody status. Results shown as mean ± SD, median (IQR) or proportion.

	No HLA Antibody n = 193	HLA Antibody n = 65		
		Non DSA n = 28	DSA n = 37	p value
Age at transplant (years)	47 ± 13	46 ± 11	44 ± 11	0.45
Donor Age (years)	44 ± 17	44 ± 17	43 ± 15	0.89
Recipient Female	53 (28%)	19 (68%)[a]	22 (60%)[a]	<0.001
Donor Female	88 (46%)	12 (43%)	20 (54%)	0.58
Cadaveric donor	118 (61%)	21 (75%)	28 (76%)	0.11
Repeat Transplant	7 (4%)	9 (32%)[a]	11 (30%)[a]	<0.001
HLA Mismatch	3 (3–4)	2 (1–4)	3 (2–4)	0.11
DGF	34 (18%)	3 (11%)	10 (27%)	0.22
IL2R-Ab induction	87 (47%)	15 (54%)	25 (68%)	0.65
Anti-T cell induction	3 (2%)	1 (4%)	2 (5%)	
Initial CNI tacrolimus	123 (64%)	16 (57%)	28 (76%)	0.26
Prior transfusion	70 (36%)	16 (57%)[a]	23 (62%)[a]	0.003
All BPAR (AMR and non-AMR)	66 (34%)	13 (46%)	25 (68%)[a]	0.001
Time to BPAR (days)	61 (47–74)	73 (55–77)	59 (53–83)	
AMR	12 (6%)	1 (4%)	11 (30%)[a,b]	<0.001
Time to AMR (months)	45 (32–60)	49	1 (0–26)[a,b]	
Non-AMR	54 (30%)	12 (44%)	14 (54%)[a]	0.027
Non-BPAR (clinical diagnosis)	21 (11%)	6 (21%)	3 (8%)	0.21

[a] Significantly different from No HLA antibody.
[b] Significantly different from Non-DSA antibody.