Complement-binding donor epitope sharing antibodies in a kidney transplant recipient

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Dear Sir,

Human leucocyte antigen (HLA) molecules have multiple epitopes and some epitopes are shared by distinct HLA antigens. The anti-HLA antibodies detected in sera from sensitised recipients are specific for epitopes shared by different HLA antigens, rather than private HLA antigens[1,2]. The Luminex single antigen-bead (SAB) assay using single recombinant HLA alleles is currently the most sensitive method for detecting HLA antibodies and also assists in determining unacceptable HLA antigens and antibody epitopes.

Complement-fixing, donor-specific HLA antibodies (DSA) are associated with antibod-mediated rejection and poor clinical outcome following kidney transplantation. Although there has been some controversy over the clinical outcome of low-level DSA and C1q positivity, the combined assay with SAB-C1q improved the assessment of complement-binding DSA in sensitised transplant patients[2]. For functional epitope analysis, the HLAMatchmaker programme can determine eplet repertoires which share mismatched eplets[3]. Here we present an interesting clinical case with respect to complement-binding antibodies against epitopes shared between donor- and non-donor HLA antigens which were determined via SAB-C1q assay and epitope analysis.

A 53-year old female patient with chronic renal failure received an ABO-compatible living kidney transplant, which was donated by her son. The patient was sensitised to HLA antigens due to prior blood transfusion and pregnancy. The patient's HLA type was A*26:01, *31:01, B*35:01, *40:02, DRB1*04:05, *08:02, DQB1*03:02, *04:01 and the donor's was A*31:01, *31:01, B*35:01, **67:01*, DRB1*04:05, **16:02*, DQB1*04:01, **05:02* (marker mismatches in italics). For epitope analysis, HLA-A, -B, -DRB1, and -DQB1 genotyping was retrospectively performed via sequence-based high resolution HLA typing. Before transplantation, T-cell complement-dependent cytotoxicity (CDC) cross-matches were positive (1:32), as were flow cytometric cross-matches. T-cell CDC with anti-human immunoglobulin was also positive. The SAB-IgG assay (Lab Screen, One Lambda, Canoga Park, CA, USA) and SAB-C1q assay were performed to confirm the donor specificity and complement-fixing capacity. Detected antibodies had a weak to moderate response against donor HLA-B*67:01 antigen (MFI=6,046) without C1q binding activity, but had a strong reaction against several non-donor-specific HLA-B antigens in both the SAB-IgG and SAB-C1q assays (Table I). Using SAB-IgM and dilution analysis, a DSA-IgM or prozone effect was ruled out. According to our Centre's desensitisation protocol (Figure 1), rituximab at a dose of 375 mg/m^2 (MabThera™; Genentech, San Francisco, CA, USA) was administered before transplantation, and plasmapheresis/immunoglobulin (100 mg/kg) therapy was administered every other day for 2 weeks with fresh-frozen plasma or albumin replacement fluids. Immunosuppressant treatment was initiated 7 days prior to transplantation with tacrolimus in combination with mycophenolate mofetil and prednisolone (Table I). After two infusions of bortezomib (1.3 mg/m^2), negative-CDC cross-matching was achieved and the patient received a kidney transplant with basiliximab induction therapy. A biopsy 4 months after transplantation, as per protocol, showed C4d-negative antibody-mediated rejection, type II. The patient had good renal function with a serum creatinine level of 1.01 mg/dL and had stable allograft function until 15 months after the kidney transplant.

DSA reactivities were monitored in post-transplantation sera. Anti-HLA antibodies, normalised mean fluorescence intensity (MFI) values and informative eplets that mismatch with the self HLA allele before and after transplantation (1 week, 4 months, and 1 year) are listed in Table I. Eplets of HLA antibodies were analysed with the HLAMatchmaker programme using the high-resolution HLA A-, B-, C-, DR-, DQ-type and SAB results. All detected antibodies with MFI values more than 3,000 were reactive against 44RE, 65QIA, 70IAQ eplets shared with donor HLA B*67:01 antigen. This is consistent with a previous report stating that the antibody producer is often exposed to multiple HLA incompatibilities, but that the specificities of

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Table I - SAB-IgG and SAB-C1q assays and cross-matching test results and desensitisation/immunosuppression protocol.

Informative eplets on reactive alleles	Alleles	Before KT		Day of KT		1 week after KT		4 months after KT		1 year after KT	
		SAB-IgG MFI	*SAB-C1q MFI*	*SAB-IgG MFI*	*SAB-C1q MFI*	*SAB-IgG MFI*	*SAB-C1q MFI*	*SAB-IgG MFI*	*SAB-C1q MFI*	*SAB-IgG MFI*	*SAB-C1q MFI*
44RE, 65QIA, 70IAQ	B*07:02	6,888	12,599	13,046	81	4,677	0	9,834	34	6,236	0
44RE, 65QIA, 70IAQ	B*81:01	6,834	11,052	11,719	70	4,429	0	9,748	64	5,394	0
44RE, 65QIA, 70IAQ	B*67:01-donor	6,046	317	10,759	0	1,597	0	3,134	0	1,221	0
44RE, 65QIA, 70IAQ	B*42:01	5,209	651	9,465	0	1,729	0	3,900	0	1,366	0
44RE, 65QIA, 70IAQ	B*55:01	5,082	192	9,226	0	1,177	0	2,683	0	863	0
44RE, 65QIA, 70IAQ	B*82:01	4,862	279	7,626	0	1,058	0	2,355	0	777	0
44RE, 65QIA, 70IAQ	B*56:01	3,684	73	8,529	0	1,241	0	2,745	0	818	0
	Self-alleles	2.3±2.8	8.3±9.6	55.5±67.6	0	24.6±11.3	0	1.3±1.5	0	0	0
Cross-match	T-cell CDC	Positive (1:32)		Negative		Negative		Negative		Negative	
	T-cell FCXM	Positive		Positive		Positive		Negative		Negative	

KT: kidney transplant; MFI: mean fluorescence intensity.

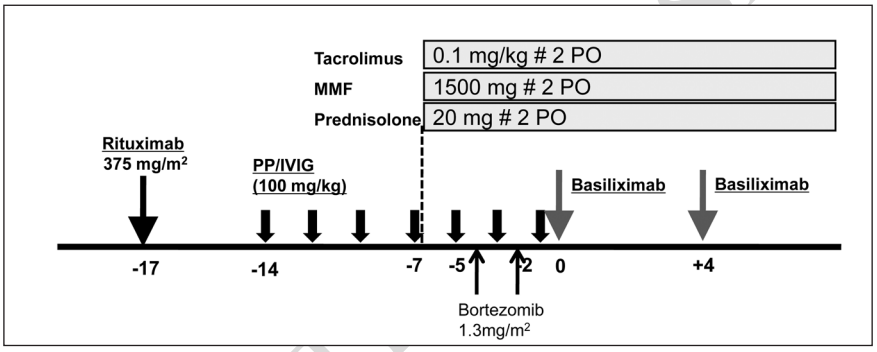

Figure 1 - Desensitization/immunosuppression protocol.
MFI: mean fluorescence intensity; KT: kidney transplantation; SAB: single antigen bead; CDC: complement-dependent cytotoxicity; FCXM: flow cytometric cross-match; PP/IVIG: plasmapheresis/immunoglobulin; MMF: mycophenolate mofetil.

the antibodies are generally limited to a few epitopes during humoral immunisation[4]. In SAB-C1q assay, DSA (anti-B*67:01) was C1q-negative, but two alleles (HLA-B*07:02, B*81:01) which share the epitopes with B*67:01 gave strong positive reactions with MFI >10,000. This finding suggests that DSA (anti-B*67:01) has negative complement-binding reactivity with a MFI value of 317 but the restricted antibodies against donor specific-epitopes (44RE, 65QIA, 70IAQ) seem to induce CDC-positive results. Our case suggests that CDC assays may be positive due to complement-fixing antibodies against non-donor antigens that share epitopes with donor antigens. Since a subset of antibodies recognising a limited number of epitopes can activate the complement, detection of immunogenic epitopes is important in a transplantation and transfusion laboratory, and the SAB assay has made it possible to determine epitopes experimentally. Despite desensitisation therapy, including bortezomib infusions, donor epitope-sharing antibodies showed increased MFI levels at the time of transplantation. The normalised MFI value of DSA (anti-B*67:01) also increased to 10,759 by SAB-IgG assay, and the flow cytometry cross-matching results remained positive. However, all detected antibodies were C1q-negative, and the CDC assay became negative. This may be due to rebound overproduction, or non-complement binding IgG returning to plasma from extravascular reservoirs.

In previous studies[5], it was reported that the presence of C1q-positive DSA was associated with a significantly greater incidence of acute allograft rejection and significantly higher probability of the presence of C4d deposition on biopsy. However, the relationship between C1q-positive donor epitope-sharing antibodies and poor graft outcome is not certain. In our case, the patient showed antibody-mediated rejection 4 months after transplantation. Persistence of antibodies against donor epitope-sharing antigens might play a role in the development of post-transplant antibody-mediated rejection. However, staining for C4d was negative at 4

months and the patient maintained a clinically stable allograft with negative cross-matching in the presence of donor epitope-sharing antibodies. This may be due to a less sensitive C4d stain on the biopsy, the effect of weaker antibodies, variation in antigen density on SAB, different bead reactivity, presence of non-HLA-DSA, transplantation in a window period without detectable antibodies or successful desensitisation with immunosuppression.

In conclusion, this case underlines the importance of complement-fixing allele screening tests in sensitised patients. It also highlights that that the presence of non-donor-specific HLA antibodies should be evaluated in the compatibility tests, in particular if they share donor-specific epitopes. However, a further prospective study is needed to confirm the association of complement-fixing epitope-specific antibodies with cytotoxic cross-matching or allograft outcome.

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