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LETTER TO THE EDITORS

Serum complement inactivation unveiled prepregnancy donor-specific HLA antibodies leading to postpartum kidney graft loss

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Dear Sirs,

Pregnancy in solid organ-transplanted patients can stimulate acute cellular rejection and formation of *de novo* donor-specific antibodies (DSA) that may cause antibody-mediated rejection (AMR) and graft loss [1,2]. Allostimulation in transplanted pregnant patients has been in some cases ascribed to HLA antigens shared between the organ donor and fetus and not expressed by the mother [3,4]. However, *de novo* DSA may also precede pregnancy and possibly lead to graft damage.

Here, we report the case of a postpregnancy graft loss due to AMR in an unsensitized young recipient of a first kidney graft, in whom prepregnancy *de novo* DSA, previously undetected and retrospectively revealed by serum complement inactivation, were the principal risk factor for the induction of graft damage after pregnancy.

An 18-year-old-female dialysis patient underwent kidney transplantation to treat renal hypodysplasia. The patient, who was unsensitized and never transfused before the transplant, was grafted with a kidney from a deceased donor. HLA mismatches with the donor are shown in Table 1a. Immunosuppression consisted of induction with anti-CD25 Mab, followed by maintenance therapy with cyclosporin A, mycophenolate mofetil and prednisone. The post-transplant period was characterized by prompt recovery of renal function and by absence of adverse events.

At 3 years post-transplant, the patient, still on triple therapy (cyclosporin A 225 mg/day, mycophenolate mofetil 1000 mg/day, prednisone 5 mg each other day), asked to plan a pregnancy. Consequently, patient sera were tested for the presence of *de novo* HLA antibodies as part of the prepregnancy counseling. HLA antibodies were analyzed by the LABScreen Mixed kit and Class I and Class II Single Antigen kit (One Lambda Inc., Canoga Park, CA, USA) [5]. Two prepregnancy serum samples resulted negative for HLA antibodies (Table 1b). The husband's HLA typing revealed the presence of three inheritable mismatch antigens shared between donor and husband, two of which were related to DQB1*06 and DQA1*01 broad specificities, respectively: donor DQB1*0603 versus husband DQB1*0602; donor DQA1*0103 versus husband DQA1*0102. The patient confirmed her willingness to plan a pregnancy and mycophenolate mofetil was substituted with azathioprine.

Forty-three months post-transplant, the patient became pregnant and underwent, due to rupture of membranes, a spontaneous premature delivery of a healthy male at 24 weeks. During this period, her renal function remained stable and good. The analysis of a serum sample, collected during pregnancy, showed the presence of a DSA against DQB1*0201 specificity, expressed by the donor only (Table 1b). The neonate's HLA typing demonstrated that all possible repeated shared mismatches were inherited (Table 1a). Seven months after the delivery, concurrently with an initial deterioration of graft function, DSA analysis detected five further DSA specific for antigens expressed by the donor only or directed against shared antigens (Table 1b). Medication nonadherence was never suspected due to regular attending of clinical visits and evidence of appropriate drug levels. Patient graft function progressed to failure in the following 22 months due to AMR not responsive to three courses of plasmapheresis associated to low-dose iv immunoglobulins and rituximab infusions. Despite these treatments, DSA MFI levels, but HLA-A*03, remained unchanged (Table 1b).

All sera were retrospectively tested with C1q binding single-antigen bead (SAB) technology [6]. The results showed that, during pregnancy, in addition to the DQB1*0201 DSA that tested positive for C1q binding, other DSA directed to DQB1*06 (reacting with both DQB1*0603 and DQB1*0602) and B*08 were detected by this assay. DQB1*0603 and B*08 antibodies were positive also in prepregnancy sera. On the first postpregnancy serum, collected before any antibody removal procedure, all DSA showed C1q binding ability (Table 1b).

Table 1. (a) HLA mismatches with the donor and shared mismatches between donor and child. (b) Donor-specific and donor/child-specific HLA antibodies and mean fluorescence intensity (MFI) values detected in the recipient at different posttransplant intervals.

(a)	HLA- A		HLA- B		HLA- C		HLA-DRB1		HLA-DQB1		HLA-DQA1	
Donor	A*01	A*03	B*08	B*38	C*07	C*12	DRB1*03	DRB1*13	DQB1*02:01	DQB1*06:03	DQA1*01:03	DQA1*05:01
Child		A*03								DQB1*06*	DQA1*01*	

		Prepregnancy		Pregnancy	Post Delivery				Graft†
(b)	Specificity	26/03/2007	02/04/2008	23/04/2009	25/11/2009	02/03/2010	17/05/2010	18/04/2011	12/01/2012
CL I SAB	A*01	neg	neg	neg	21 900	20 000	19 000	14 700	9600
	A*03	neg	neg	neg	9300	neg	neg	neg	neg
	B*08	neg (7349)	neg (7439)	neg (8037)	7800	2500	2000	4600	neg
CL II SAB	DQB1*0201	neg (6902)	neg (4671)	11 000	22 500	19 428	17 128	16 800	5900
	DQB1*0602	neg	neg	neg (12 460)	23 100	19 000	neg	16 000	2000
	DQB1*0603	neg (1280)	neg (1181)	neg (20 722)	23 300	20 255	neg	17 000	4000
CL I C1q	A*01	neg	neg	neg	23 700	25 414	24 200	24 200	na
SAB	**A*03**	neg	neg	neg	3500	neg	neg	neg	na
	B*08	1600	702	2000	1300	neg	neg	neg	na
CL II C1q	DQB1*0201	neg	neg	17 750	24 300	23 473	23 850	neg	na
SAB	**DQB1*06**02	neg	neg	16 200	25 500	22 516	22 300	16 700	na
	DQB1*0603	neg	542	24 000	24 400	24 051	25 500	24 600	na

SAB: single-antigen bead assay. Positivity cut –off: MFI values above 1000.
C1q-SAB: C1q binding assay. Positivity cutoff: MFI values above 500.
HLA antibodies specific for antigens shared between donor and child are indicated in bold.
MFI values obtained after serum heat treatment are reported in brackets; negative results are not reported.
*For DQ loci, shared mismatches were considered also at a two digit typing level.
†Eluates were obtained from tissue fragments of explanted kidney graft; na: not applicable.

In post-therapy samples, A*03 and B*08 C1q binding DSA appeared completely downmodulated. The patient underwent kidney removal 75 days after return to dialysis, due to hematuria and hypertension. Acid pH eluates, obtained from tissue samples of the explanted graft, were analyzed for the presence of intragraft DSA with the SAB technology [7]. The results showed that those DSA which consistently displayed high MFI levels in the serum could be detected within the graft (Table 1b). The detection of additional DSA by C1q-SAB technology, where sera are heated before testing, prompted us to re-analyze sera after complement inactivation by heating serum for 30 min at 56 °C. This modification allowed to detect B*08, DQB1*0201 and DQB1*0603 DSA in prepregnancy sera not previously observed with the standard SAB technique (Table 1b), thus demonstrating that a donor-specific immune response was already present.

The above finding suggests that standard SAB analysis could underestimate, or even miss, the presence of HLA antibodies. This phenomenon has been recently attributed to a complement inhibitory interference in the SAB assay, likely due to a quenching effect exerted on the bead surfaces by C4 and C3 activation products [8,9]. The presence of a complement dependent hook effect in our SAB assays is supported by the demonstration that DSA detected after heat inactivation, with the exception of DQB1*0201 antibody, were endowed with C1q binding capability. In our case, the risk, represented by the presence of prepregnancy DSA, was likely magnified by the shift from MMF to azathioprine, and the involvement of repeated mismatch effect seemed not significant. Indeed, with the exception of a transient antibody against HLA-A3 (plausibly stimulated by a common epitope on HLA-A1), all the antibodies specific for shared mismatches were present in the prepregnancy and no antibodies against other donor and child mismatches were found. The delivery of the child rather than the pregnancy per se seems to represent an other relevant event as demonstrated by the observation that the graft function remains good prepregnancy (when DSA were already present) and during the pregnancy when the MFI levels of DSA were maintained or raised. Accordingly, DSA testing after the critical steps of immunosuppression change and delivery should be performed.

In conclusion, we believe that the policy of HLA Ab screening should be suggested also in low immunological risk patients and included in counseling protocols. In this context, SAB serum analysis after treatments aimed to inhibiting complement interference (i.e., heat inactivation or ethylenediaminetetraacetic acid) could help define immunological maternal risk categories.

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Conflicts of interest

All the authors have read and approved the manuscript and have no conflict of interest to declare.

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