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Human leukocyte antigen epitope analysis to assess complement- and non-complement-binding donor-specific antibody repertoire in a pediatric heart transplant recipient

Adriana Zeevi [a,*], Marilyn Marrari [a], Brian Feingold [a,b], Steven Webber [a,b], Rene J. Duquesnoy [a]

[a] University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA
[b] Pediatric Cardiology, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA 15224, USA

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ABSTRACT

This case report summarizes the spectrum of anti–human leukocyte antigen (HLA) antibody reactivity determined by single-allele Luminex immunoglobulin G and C1q binding assays before transplant, during an episode of antibody-mediated rejection (AMR), and following treatment in a sensitized pediatric heart transplant (Tx) recipient. We were able to discriminate between complement- and non-complement-binding epitope-specific antibodies present against a single donor antigen (HLA-A2) during the progression of AMR and its resolution. Our findings illustrate the usefulness of determining antibody specificities against epitopes using various Luminex-based assays.

1. Introduction

Solid-phase antibody (Ab) detection assays with single human leukocyte antigen (HLA) alleles have improved the analysis of antibody patterns and the identification of donor-specific antibody (DSA) in sensitized transplant recipients [1]. Luminex immunoglobulin G (L-IgG) detects both cytotoxic and noncytotoxic DSA. A Luminex-based assay that detects C1q binding to HLA-Ab (L-C1q), and thus complement activation, has recently become available [2,3]. The presence of L-C1q Ab, but not L-IgG, before and immediately after heart transplantation in a cohort of 11 children was predictive for the development of antibody-mediated rejection (AMR) [2]. The significance of pretransplant complement-fixing antibodies has also been addressed by Rose and Smith in a large cohort of adult heart transplant recipients [4]. Using a different complement-binding assay for C4d on Luminex beads, they demonstrated that the ability to fix C4d was strongly associated with poor allograft survival after heart transplantation [4].

The HLAMatchmaker program determines HLA antibody reactivity using an algorithm to assign to each HLA antigen a string of structurally defined "eplets" that represent potential epitopes consisting of polymorphic amino acids located within a 3-Å radius on the surface of the molecule [5,6]. The program first determines the eplets that are present on the patient HLA type using high-resolution (4-digit) HLA types. If these are not available, a converter helps the user assign the most likely 4-digit alleles based on the patient population. When used for antibody analysis, HLAMatchmaker then determines for each allele in the Luminex panel the eplets that are different from those on the patient's HLA antigens. Eplets on alleles that yield negative reactions are considered acceptable mismatches and are eliminated from the analysis. The remaining eplet repertoires on alleles with positive reactions are compared to determine whether they share any mismatched eplets that may explain the antibody reactivity pattern. The Excel spreadsheet–based program is available at http://www.HLAMatchmaker.net.

In a sensitized pediatric heart Tx recipient who was transplanted across positive T- and B-cell cytotoxic crossmatches, we conducted HLAMatchmaker analyses of serum reactivity to determine which epitopes, referred to as eplets, were recognized by the patient's antibodies detected by L-IgG and L-C1q single-allele assays [5,6]. We compared the repertoire of DSA-associated eplets that bound complement or lacked complement-binding activity before and after treatment of AMR and correlated our findings with clinical outcome.

* Corresponding author.
E-mail address: zeevia@upmc.edu (A. Zeevi).

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Table 1
Epitope-specific antibody reactivity patterns Patient HLA type: A*03:01, -; B*07:02, B*38:01; C*07:02, C*12:03 Immunizer HLA type: A*02:01,A*11:01; B*35:01,B*40:01; C*03:04,C*04:01

		Pre-Tx		10 days post-Tx		30 days post-Tx	
		No C1q MFI	With C1q MFI	No C1q MFI	With C1q MFI	No C1q MFI	With C1q MFI
	Negative control	253	37	123	76	138	166
	Positive control	21,961	N/A	18,068	N/A	12,646	N/A
	Self-alleles	223 ± 219*	7 ± 15	13 ± 19	3 ± 5	378 ± 198	0 ± 0
Informative eplets on reactive alleles	Allele						
138MT, 62GE, 127K, 193AV	A*02:01-donor	19,362	14,150	12,726	23,516	12,341	0
138MT,62GE,127K,193AV	A*02:03	20,330	6,587	11,217	24,007	13,820	0
138MT,62GE,127K,193AV	A*02:06	20,011	14,330	12,754	24,152	15,689	0
138MT,127K,193AV	A*68:01	17,354	15,927	10,691	24,310	11,013	0
138MT,127K,193AV	A*68:02	19,264	15,148	12,576	23,889	11,814	0
138MT,127K,193AV	A*69:01	21,464	7,079	16,550	23,503	13,813	0
127K	A*23:01	4,751	17	16,823	3,056	9,763	0
127K	A*24:02	7,892	7	15,747	16,744	9,791	0
127K	A*24:03	8,366	14	15,512	17,873	8,582	0
62GE	B*57:01	12,862	36	17,404	17,403	603	0
62GE	B*57:03	12,516	21	18,487	15,914	1,215	0
62GE	B*58:01	10,991	23	19,103	10,202	312	0
144QL,41T,45RMA	B*13:01	1,308	20	18,289	6,384	4,806	0
144QL,41T,45RMA	B*13:02	1,388	37	18,910	5,887	6,336	0
193AV	A*25;01	809	0	7,040	0	1,321	0
193AV	A*26:01	920	6	6,082	0	841	0
193AV	A*29:01	894	106	5,006	59	552	75
193AV	A*29:02	607	17	4,739	0	503	0
193AV	A*31:01	658	9	4,580	0	448	0
193AV	A*32:01	318	7	4,143	0	363	0
193AV	A*33:01	524	3	3,694	0	370	0
193AV	A*33:03	342	1	4,063	0	397	0
193AV	A*34:01	1,085	14	4,683	0	689	0
193AV	A*34:02	237	0	4,529	0	323	0
193AV	A*43:01	1,105	1	4,952	0	473	0
193AV	A*66:01	834	4	6,264	0	1,225	0
193AV	A*66:02	719	0	3,616	0	641	0
193AV	A*74:01	116	0	4,008	0	166	0
41T	(10 alleles)	810 ± 466	22 ± 18	14,421 ± 2,827	108 ± 66	941 ± 560	0 ± 0
44RMA	(7 alleles)	778 ± 622	11 ± 8	9,965 ± 4,354	43 ± 54	1,775 ± 944	0 ± 0
Other	(47 alleles)	621 ± 471	20 ± 25	248 ± 500	10 ± 17	458 ± 286	2 ± 14

2. Case description

A 14-year-old blood group O female who received an HLA-mismatched primary heart Tx at age 6.5 years presented with acute heart failure. The patient typed as HLA-A3,-; B7,38; Cw7,w12; DR8,15, DQ3,6, DR51,- and the first donor typed as HLA-A1,3; B7,8; DR15,17; DQ1,2. After 7 weeks of escalating intravenous inotropic support, she underwent re-Tx from an HLA-mismatched donor who typed as A2,11; B35,60; Cw3,w4; DR11,15; DQ1,7; DR51,52. Serum drawn before the second Tx exhibited an anti-HLA-A2 antibody pattern (Table 1). Although her first donor did not type as HLA-A2, the patient had received non-HLA-matched homograft tissue for augmentation of the aortic arch before her first Tx. Because of the presence of DSA before her second transplant, the patient was treated with a 1.5-vol plasma exchange preoperatively and continued on an empiric regimen of plasmapheresis for 5 consecutive days postoperatively. The T- and B-cell complement-dependent cytotoxicity crossmatches with pre-exchange, pretransplant serum were both strongly positive (1:32 and ≥1:8, respectively); however, both crossmatches were negative with postexchange, pretransplant serum. Immunosuppression consisted of induction therapy with thymoglobulin daily for 5 days and maintenance therapy with tacrolimus, mycophenolate mofetil, and corticosteroids (initially given intravenously as methylprednisolone). Intravenous immunoglobulin (IVIG) was given after the fifth postoperative plasmapheresis was completed.

Early echocardiograms indicated normal allograft function and the initial endomyocardial biopsy on day 7 postoperatively demonstrated diffuse C4d immunostain positivity without histologic features of acute cellular rejection or AMR. Follow-up biopsy on day 14 indicated AMR with myocyte injury, interstitial edema, intracapillary neutrophils, hemorrhage, and ongoing diffuse capillary C4d staining. The patient was treated with pulse intravenous methylprednisolone (10 mg/kg × 5 doses), IVIG (1 g/kg × 2 doses), and rituximab (375 mg/m^2 × 3 doses at weekly intervals).

Subsequent biopsies continued to demonstrate histologic features of AMR along with diffusely positive C4d immunostaining, although hemodynamics, cardiac output measurements, and echocardiography remained normal. At 13 months after transplant, C4d was no longer detected on the allograft. The patient remains well with normal graft function 24 months after re-Tx.

3. Results and discussion

In Table 1 we compare the results of the HLAMatchmaker analyses of the pre-Tx, 10 days post-Tx, and 30 days post-Tx serum samples as determined in the L-IgG and L-C1q binding assays (LabScreen, One Lambda, Canoga Park, CA). The Luminex results are presented as the normalized trimmed mean of mean fluorescence intensity (MFI) values. Because the high-resolution types were not available, the patient and donor HLA types were converted to the most likely 4-digit alleles using the converter in the HLAMatchmaker program.

For the L-IgG assay with pre-Tx serum, the average MFI value of the patient's 5 self-alleles was 223 ± 219. Twelve alleles gave strong positive reactions and are listed along with their MFI value and the informative eplets that are mismatches for the patient alleles (Table 1). These positive reactions can be explained by antibody reactivity toward 3 eplets on donor HLA-A2: 62GE (shared by HLA-A2, -B57, -B58), 127K (shared by HLA-A2, -A23, -A24, -A68,