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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

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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.

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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,

-A69), and 138MT (shared by HLA-A2, -A68, -A69). In addition, weak reactivity toward 2 HLA-B13 alleles that share third-party eplets 144QL, 41T, and 44RMA was also detected. Results are also presented for 3 other groups of alleles (Table 1). The first group consisted of alleles that share eplet 193AV with HLA-A2, -A68, and -A69: -A25, -A26, -A29, -A31, -A32, -A33, -A34, -A43, -A66, and -A74. For the other 2 groups the mean MFI of the alleles that share with HLA-B13 either eplet 41T (HLA-B60, -B61, -B41, -B44, -B45, -B47, -B49, -B50) or eplet 44RMA (HLA-B46, -B62, -B63, -B75, -B76, -B77) are given. In the pre-Tx L-IgG assay, these eplet groups had MFI values similar to those of 47 other alleles not listed (Table 1). The mean MFI of all these groups was 3- to 4-fold higher than that of self-alleles ($p < 0.01$).

As indicated in the second column of Table 1, the L-C1q assay had a more limited pattern of reactivity. Only 6 alleles (HLA-A2, -A68, -A69) were positive and they all share eplet 138MT. Because HLA-A23, -A24, -B57, and -B58 were negative in this assay, we assume that the serum lacks C1q binding reactivity toward eplets 62GE and 127K. Furthermore, the C1q reactivity toward all other allele groups was also negative.

In this example we illustrate how HLAMatchmaker can explain the functional diversity of HLA-specific epitopes determined by the presence or absence of complement-binding reactivity on HLA-typed panels. In this case, sensitization to donor HLA-A2 antigen was potentially induced by the homograft tissue and not by the first donor. As expected by the positive complement-dependent cytotoxicity T and B crossmatch results, the pretransplant serum contained complement-binding DSA determined by the L-C1q assay. However, using eplet analysis, we could demonstrate that the observed restricted reactivity pattern with the C1q assay was toward only 1 of the 3 HLA-A2 epitopes positive with the L-IgG.

Despite receiving intraoperative plasmapheresis and IVIG therapy, the patient developed AMR within 10 days posttransplant. This clinical outcome correlated with increased alloantibody reactivity in both assays. During AMR, the L-IgG-positive reactions included the 14 alleles that were positive in the pretransplant serum, but also 33 additional alleles that share eplets 193AV, 144QL, 41T, and/or 44RMA (Table 1, third column of MFI results). This reactivity could be caused by the original HLA-A2 and -B13 sensitization events because the level of antibody in the pretransplant serum may have been below the level of detection. The 41T epitope is also shared by the donor B60 allele.

The C1q reactivity during AMR (Table 1, fourth column) was different from the L-IgG pattern and was restricted to alleles that share the 3 HLA-A2 eplets originally identified by L-IgG assay with the pre-Tx serum (62GE, 127K, 138MT) and to the eplet 144QL, which is unique for HLA-B13. The observed reactivity by L-IgG toward alleles that share eplets 193AV, 41T, and 44RMA was absent in the L-C1q binding assay.

Persistent anti-HLA-A2 DSA with alleles that share either eplet 138MT or 127K was detected by L-IgG following 1 month of treatment; MFI values ranged from 8,000 to 12,000 in the L-IgG assay, similar to the pre-Tx levels. Reactivity was also seen toward the unique HLA-B13 eplet 144QL. However, alleles that share eplets 62GE and 193AV with HLA-A2 exhibited low MFI values, similar to those seen for the "other alleles." Although the HLA-B13-related 41T and 44RMA eplet groups exhibited low MFI values, the values were significantly higher than the mean of 458 ± 286 seen for the "other alleles" group (941 ± 560 [$p < 0.05$] and 1,775 ± 944 [$p < 0.03$], respectively). All L-C1q results were negative 30 days post-Tx (Table 1, last column).

We also evaluated pre- and posttransplant the strength of DSA toward the various eplet groups by titration of the sera (neat, 1:4, 1:16; Fig. 1a–c). The anti-HLA-A2 reactivity associated with eplet 138MT (A2 and A28 allele groups) demonstrated persistent strong MFI (>15,000) at 1:16 dilution pre-Tx and 10 days post-Tx during the AMR episode (Fig 1a, 1b) and these high-titer DSAs were demonstrated to be C1q reactive (Table 1). However, 1 month posttreatment when the titer of these DSAs dropped, the C1q assay became negative (Fig 1c, Table 1).

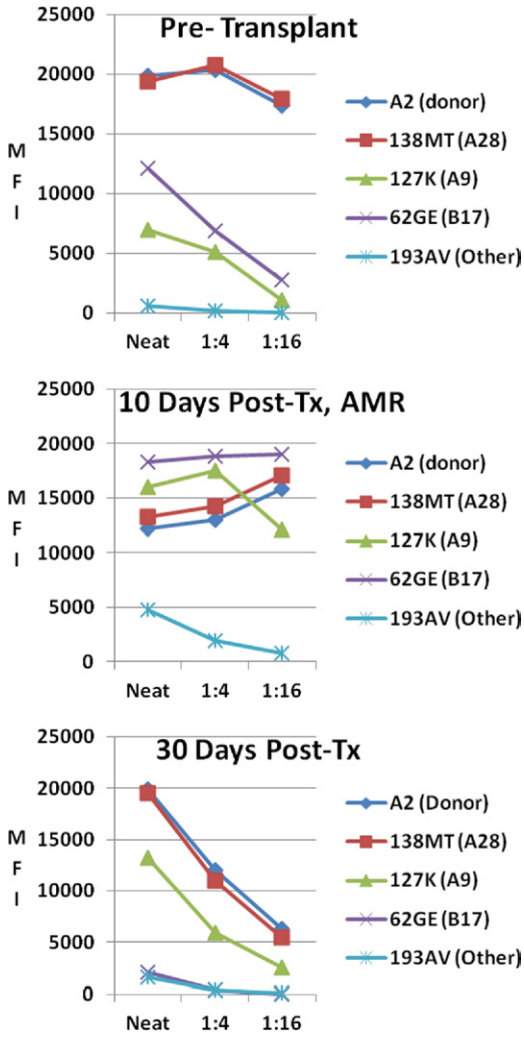

Fig. 1. Titration of anti–human leukocyte antigen (HLA)-A2 epitope-specific antibodies (a) pretransplant, (b) 10 days posttransplant (post-Tx), and (c) 30 days post-Tx. Informative HLA alleles for given epitopes are presented in parentheses. Mean fluorescence intensity (MFI) values are normalized. AMR, antibody-mediated rejection.

The MFI values for alleles sharing the HLA-A2-related eplets 127K and 62GE (A9 and B17 allele groups) were much lower at 1:16 dilution pretransplant and at 1 month post-Tx compared with that during the AMR course (Fig. 1). Of note, C1q reactivity with alleles bearing 127K and 62GE eplets was associated with higher titer only observed during the AMR episode.

4. Conclusions

In this case we illustrate the functional diversity of HLA-A2 DSA reactivity patterns obtained with L-IgG and L-C1q assays and determined the eplet repertoire using HLAMatchmaker. The association of C1q binding with high-titer DSA may reflect the necessity of a certain density of anti-HLA antibody molecules on the surface of the cell/bead to bind the complement component. The relationship between high-titer HLA antibody and C1q-binding ability was also seen with HLA-B13 alleles (with unique eplet 144QL); the MFI values remained high after dilution in the AMR serum and were also C1q positive (Table 1). However, serum reactivity toward al-

leles that share eplets 193AV with HLA-A2 and 41T and 44RMA with HLA-B13 (including donor HLA-B60) exhibited a low titer during the AMR and declined very quickly following therapy. These alleles also were largely negative in the L-C1q assay (Table 1).

Lack of DSA with C1q binding 1 month posttherapy correlated with improved clinical outcome despite persistent C4d-positive staining up to 1 year posttransplant. The patient experienced 2 acute cellular rejection episodes in the second year with detection of low-level DSA, C1q negative, and negative C4d in the biopsy (data not shown).

In summary, our findings illustrate the usefulness of determining antibody specificities against epitopes from serum reactivity patterns with single alleles in the various Luminex-based assays. The complement-binding reactivity of certain epitopes was associated with the AMR process and complement deposition in the tissue. Furthermore, the efficacy of intervention corresponded with the loss of complement-binding reactivity and low titer of epitope-specific DSA.

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