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Why can sensitization by an HLA-DR2 mismatch lead to antibodies that react also with HLA-DR1?

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ABSTRACT

HLAMatchmaker is a matching algorithm that can be used to characterize antibodies specific for structurally defined epitopes. Under the auspices of the 15th International Histocompatibility Workshop, we are conducting a multilaboratory collaborative project to characterize these epitopes and also to determine how often they induce specific antibodies in patients with rejected kidney transplants. This report addresses the reactivity of post-allograft nephrectomy sera tested for DRB antibodies with Luminex assays using single alleles. This analysis was performed for 19 informative kidney transplant cases contributed by 13 laboratories worldwide. There were 11 cases with a single DR2 mismatch (DR15 or DR16), and nine of them (82%) showed antibodies with both DR2 and DR1. Although these antigens might share an epitope recognized by these antibodies, this interpretation is incorrect. The HLAMatchmaker analysis offers a clearly different explanation that involves antibodies induced by DR51, which commonly associates with DR2. DR51 has an epitope defined by the 96EV eplet, which is also present on DR1 but no other DR antigen. This means that the reactivity with DR51 and DR1 reflects the presence of 96EV-specific antibodies. Conversely, we analyzed eight patients sensitized by a single DR1 mismatch that has no associated DR51. All of these patients reacted also with DR51, and this could be explained only by antibodies against the shared 96EV eplet. These findings demonstrate that 96EV represents a highly immunogenic epitope that can induce cross-sensitization between antigens encoded by the different DRB loci, and also that DR51 is important in determining DRB mismatch acceptability of potential donors.

This analysis has also demonstrated that antibody responses are restricted to a few epitopes on these immunizing DR antigens. For DR2 they are 142M3 (unique for DR2), 71QAA (shared with DB5*02), and 96QV (shared with DR10). DR51 mismatches appear to have three immunogenic eplets: 96EV (shared with DR1), 108T3 (unique for DR51), and 40HFD (shared with DR9). Immunogenic eplets on DR1 are 12LKF2 (unique for DR1), 14FEH (shared with DR9 and DR10), and 25HRL (shared with DR10).

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1. Introduction

HLA antibodies cause allograft rejection and decrease organ transplant survival. Sensitive assays such as Luminex with single alleles permit a detailed analysis of antibody specificity patterns to assess HLA mismatch acceptability of potential donors. An important component is the determination of the epitope repertoire on the HLA molecular surface because this information may lead to a more efficient epitope-based matching algorithm aimed to control antibody-mediated rejection.

HLAMatchmaker is a structurally based matching program that considers each HLA antigen as a string of epitopes represented by short linear sequences involving polymorphic amino acid residues (originally referred to as triplets) in antibody-accessible positions [1]. The eplet version applies the concept developed from molecular modeling of crystallized antigen-antibody complexes, that func-

tional epitopes are represented by patches of surface-exposed non-self amino acid residues surrounded by residues within a radius of approximately 3 Ångstroms [2]. These patches are referred to as "eplets"; many of them are short linear sequences common to triplets, but others have residues in discontinuous sequence positions that cluster together on the molecular surface. The eplet version of HLAMatchmaker permits a more complete assessment of the epitope repertoire.

Many sensitized patients have antibodies induced by a transplant, and a detailed analysis of antibody specificity patterns provides a better understanding of the humoral immune response to mismatched HLA antigens of the transplant donor. Serum antibodies are more readily detectable after the transplant has been removed because allograft tissue can absorb circulating donor-specific HLA antibodies. Sera from patients from whom the rejected kidney transplant had been removed have antibodies specific for a restricted number of HLAMatchmaker defined epitopes on immunizing donor HLA antigens [3].

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During humoral immunization, the antibody producer is often exposed to multiple HLA incompatibilities but the specificities of the antibodies are generally limited to a few epitopes. Under auspices of the 14th and 15th International Histocompatibility Workshops we initiated a multilaboratory collaborative project to characterize these epitopes and also how often they induce specific antibodies in patients with rejected kidney transplants. The latter would provide an assessment of the epitope immunogenicity. The 14th Workshop project has generated preliminary information about class I epitope immunogenicity [4].

The Luminex assay with single HLA alleles offers new opportunities to analyze HLA antibody reactivity patterns in much more precise detail. HLAMatchmaker is a useful tool to determine antibody specificities not only against epitopes on HLA-A, B, C antigens [2] and even MICA [5] but also on class II antigens encoded by HLA-DRB1, DRB3/4/5, DQB, DQA, DPB, and DPA loci [6,7].

More than 25 laboratories worldwide are participating in the 15th Workshop project on epitope immunogenicity. Approximately 150 informative allograft nephrectomy cases have been submitted so far, and many of them have yielded interesting information that have led to a better understanding of antibody recognition of HLA epitopes. As an example, this report describes the sensitization to DR2 (DR15 or DR16) mismatches and the analysis of antibody reactivity with DR2 and other DR antigens in the Luminex panel. Almost all sera from DR2-sensitized patients reacted also with DR1. Although an obvious interpretation could be that DR1 and DR2 might have a shared epitope, the HLAMatchmaker analysis clearly shows a different pattern of epitope sharing.

2. Subjects and methods

2.1. Patients and sera

This analysis was done on sera from patients from whom their rejected kidney transplants had been surgically removed. This study addresses antibodies induced by a single DR2 mismatch, and 13 laboratories participating in the 15th Workshop project had submitted 19 informative allograft nephrectomy cases (Table 1). HLA typing of patients and donors was performed by the contributing laboratories by standard serologic and/or molecular methods. Serum samples had been drawn an average of 89 days after allograft nephrectomy (range, 14–313 days). This study was performed on nine DR15 and two DR16 antigen mismatches, and for comparative analysis it also included eight DR1 mismatches.

2.2. Serum screening for HLA antibodies

This was done with Luminex assays using single HLA allele kits supplied by two commercial vendors (One Lambda Inc., Canoga Park, CA; Tepnel Life Codes Corporation, Stamford, CT) according to the manufacturers' instructions. In brief, an aliquot of a mixture of Luminex microspheres, each coated with a single antigen, was incubated with a small volume of test serum sample and washed to remove unbound antibody. Anti-Human IgG antibody conjugated to phycoerythrin (PE) was added and after incubation the bead mixture was diluted for analysis with the LABScan 100 instrument (Luminex Corp., Austin, TX), and the reactivity was determined with the manufacturer's software. The presence of antibody was determined by comparing the median fluorescence intensity (MFI) of the beads containing the individual antigens to the signal intensities of positive and negative control beads included in the bead mixture. Assignment of positive reactions considered also the MFI values with self-alleles.

2.3. HLAMatchmaker analysis of antibody reactivity with single allele panels

HLAMatchmaker views each HLA antigen as a string of short amino acid sequences called eplets in antibody-accessible positions; they are considered key elements of epitopes that can induce the formation of specific antibodies [2,6]. Each eplet is assigned a position number in the amino acid sequence and the polymorphic residues within a radius of approximately 3 Ångstroms; this notation does not use monomorphic residues. Amino acid residues are marked with the standard letter code. For instance, the DRB eplet 40HFD is in sequence position 40 and has three polymorphic residues: histidine (H) in position 16, phenylalanine (F) in position 40, and aspartic acid (D) in position 41. Several eplets are listed with one or two residues (for instance 120N and 96QV) because their neighboring residues are the same on all DRB chains, and they are therefore not shown. Table 2 represents a list of eplets on DR1, DR2, and DR51 alleles tested in the Luminex panel.

HLAMatchmaker applies two principles: (1) each HLA antigen represents a distinct string of structurally defined epitopes as potential immunogens that can induce specific antibodies, and (2) patients cannot make antibodies against epitopes that are expressed by their own HLA molecules [1]. The algorithm assesses donor-recipient compatibility through intralocus and interlocus comparisons, and determines what epitopes on mismatched HLA

Table 1

Allograft nephrectomy cases with DR2 and DR1 mismatches contributed by participants in the 15th International Histocompatibility Workshop project on HLA epitope immunogenicity

Case	Contributor	Location	Recipient DR antigens	Donor DR mismatch
ADEL-1	James McCluskey	Adelaide, Australia	DR8, DR13	DR15
ADEL-2	James McCluskey	Adelaide, Australia	DR4, -	DR15
AV	Mary Younie	Bristol, UK	DR8, DR9	DR15
EM	Adriana Zeevi	Pittsburgh, USA	DR8, DR13	DR15
HC	Amy Hahn	Albany, USA	DR4, DR17	DR15
ND11	Constanze Schönemann	Berlin, Germany	DR4, DR7	DR15
RM 70532482	Andrew Lobashevsky	Indianapolis, USA	DR8, DR17	DR15
SA	Silvia Chrenova	Bratislava, Slovakia	DR4, DR13	DR15
ZS	Sandra Nehlsen-Cannarella	Detroit, USA	DR11, DR17	DR15
2910	Agathi Vamavidou	Nicosia, Cyprus	DR4, DR11	DR16
POL-8790	Marilyn Pollack	San Antonio, USA	DR8, DR17	DR16
4765	Maria Gerbase-DeLima	Sao Paulo, Brazil	DR4, DR7	DR1
290544	Tsuyoshi Sato	Sapporo, Japan	DR4, DR8	DR1
695723	Tsuyoshi Sato	Sapporo, Japan	DR12, DR14	DR1
ECU-1	Lorita Rebellato	Greenville, USA	DR11, DR13	DR1
MC 05048330	Andrew Lobashevsky	Indianapolis, USA	DR8, DR17	DR1
ND4	Constanze Schönemann	Berlin, Germany	DR4, DR13	DR1
SM	Amy Hahn	Albany, USA	DR4, DR14	DR1
VS	Silvia Chrenova	Bratislava, Slovakia	DR7, DR14	DR1

Table 2

List of eplets on DR1, DR2, and DR51 and their sharing with other DRB alleles in the Luminex panel

Eplet	Amino acid residues	Eplet-carrying alleles in Luminex panel
6C	6C	DRB5*0202
12LKF ^a	11L/12K/13F	DRB1*0101/02/03
14FEH	13F/14E/16H	DRB1*0101/02/03,*0901,*1001
25HRF	16H/25R/26F	DRB1*0302/03,*0401/02/03/04/05,*1101,*1301/03,*1401,*1501/02,*1601/02, B3*0201/02,*0301,B5*0101,*0202
25HRL	16H/25R/26L	DRB1*0101/02/03,*1001
26KFD	12K/26F/28D	DRB1*0401/02/03/04/05,*1501/02,*1601/02
26RL	25R/26L	DRB1*0101/02/03,*1001,*1201/02
31QDIY	10Q/30D/31I/32Y	DRB5*0101
31QGIY	10Q/30G/31I/32Y	DRB1*0901, B5*0202
32FYN	31F/32Y/33N	DRB1*0701,*0801,*1101,*1303,*1501/02,*1601/02
32IYN	31I/32Y/33N	DRB1*0101/02/03,*0901, B4*0101/03, B5*0101,*0202
33LYNQ	27L/32Y/33N/34Q	DRB1*0101/02/03,*0701,*0801,*0901,*1101,*1303,*1501/02,*1601/02, B4*0101/03, B5*0101,*0202
40EFD	28E/40F/41D	DRB1*0101/02/03,*0302/03,*0701,*1201/02, B3*0201/02
40HFD	28H/40F/41D	DRB1*0901, B5*0101,*0202
47DFR	28D/47F/48R	DRB1*0301,*1101,*1301,*1501/02
47EYR	28E/47Y/48R	DRB1*0101/02/03,*0302/03,*0701,*1001, B3*0201/02,*0301
48FR	47F/48R	DRB1*0301,*1101,*1201/02,*1301,*1501/02
67FR	67F/71R	DRB1*0801,*0901,*1101,*1201,*1601,B5*0101
67LR	67L/71R	DRB1*0101/02,*0403/04/05,*1001,*1401,B4*0101/03
70FDRA	67F/70D/71R/73A	DRB1*0801,*1101,*1202,*1601, B5*0101
70LDRA	67L/70D/71R/73A	DRB1*1602
71DRA	70D/71R/73A	DRB1*0801,*1101,*1201/02,*1601/02, B5*0101
71QAA	70Q/71A/73A	DRB1*1501/02, B5*0202
71QRA	70Q/71R/73A	DRB1*0101/02,*0403/04/05
73AADT	73A/74A/76D/77T	DRB1*0101/02/03,*0401/02/03/04/05,*1001,*1101,*1201/02,*1301/03, *1501/02,*1601/02, B5**0101,0202
74DRAA	70D/71R/73A/74A	DRB1*1101,*1201/02,*1601/02, B5*0101
74QRAA	70Q/71R/73A/74A	DRB1*0101/02,*0404/05
81HA	81H/85A	DRB1*0102,*1201/02, B5*0202
85VV	85V/86V	DRB1*0301/03,*0402/03/04,*1301,*1401,*1501,B3*0201,*0301,B4*0101/03
98EV	98E/180V	DRB1*0101/02/03, B5*0101,*0202
98QV	98Q/180V	DRB1*1001,*1501/02,*1601/02
98KN	98K/120N	DRB1*1001, B4*0101/03, B5*0101,*0202
98KS	98K/120S	DRB1*0101/02/03,*0301/02/03,*0801,*1101,*1201/02,*1301/03,*1401,*1501/02,*1601/02
108T ^b	108T	DRB5*0101,*0202
120N	120N	DRB1*0401/02/03/04/05,*1001, B4*0101/03, B5*0101,*0202
120S	120S	DRB1*0101/02/03,*0301/02/03,*0701,*0801,*0901,*1101,*1201/02, *1301/03,*1401,*1501/02,*1601/02, B3*0101,*0201/02,*0301
135S	135S	DRB4*0101, B5*0101
140A	140A	DRB1*0101/02/03,*0701,*0901,*1501/02,*1601/02, B3*0101,*0201/02, B4*0101/03, B5*0101,*0202
142M ^c	142M	DRB1*1501/02,*1601/02
149Q	149Q	DRB1*0101/02/03,*0401/02/03/04/05,*0701,*0901,*1001,*1501/02,*1601/02, B3*0101,*0201/02, B4*0101/03, B5*0101,*0202

^aRepresents 12LKF and 31QCIY.^bRepresents 12DKY, 104AR, and 108T.^cRepresents 12PKR, 133L, and 142M.

molecules are different or shared between donor and patient. This analysis considers each donor HLA antigen mismatch toward the entire HLA phenotype of the recipient.

The DR,DQ version of the HLA Matchmaker antibody analysis program can be downloaded free of charge (<http://HLAMatchmaker.net>). The analysis can be performed in six easy steps, which are outlined below.

First, copy the alleles for the Luminex kit used for testing to the "Panel" worksheet; the program includes sheets with descriptions of single allele panels in commercial vendor kits.

Second, enter the HLA types of antibody producer and immunizer on the "Enter Data" sheet. The HLA types need to be entered as high-resolution (four-digit) alleles. If unavailable, the serologic level HLA antigens need to be converted to four-digit types that can be estimated from the most common alleles and their linkage to other alleles in various racial/ethnic groups [8–10]. The program contains a sheet with allele frequencies.

Third, enter the serum reactivity with the panel. The easiest way is to copy the median MFI values from the .csv files generated by the commercial vendor's Luminex data analysis programs and paste them into the reaction column of the "Enter Data" sheet.

Fourth, note that the program automatically calculates the average MFI for the self-alleles of the antibody producer. Determine the cut-off value for negative reactions from the MFI with self values and negative controls, and record this value in the appropri-

ate cell on the "Enter data" sheet. The program then automatically shows all alleles giving negative reactions with serum.

Fifth, record the negative alleles into dedicated boxes on the "Enter Data" sheet; as this is done the program removes their corresponding eplets from the alleles of the immunizing donor and the Luminex panel. Each reactive allele shows the remaining mismatched eplets, and a panel analysis can determine which eplets are shared with the immunizing donor and therefore might be recognized by patient's antibodies.

Sixth, record these reactive eplets in dedicated boxes on the "Enter Data" sheet. A separate "Results" sheet shows information about the antibody specificity pattern and the identification of acceptable and unacceptable mismatches.

3. Results

This study addresses the antibody response to a DR2 mismatch. Table 3 shows the results of the HLA Matchmaker analysis of Luminex data on an allograft nephrectomy case contributed by Andrew Lobashevsky at the Indianapolis Transplant Center.

This patient, who types as DR8, DR17 had rejected a kidney from a deceased donor with a DR15 mismatch. High-resolution typing showed the following result: DRB1*0301, DRB1*0801, and we determined that the patient most likely typed as DRB3*0101. The donor was a single DRB1*1501 mismatch with six mismatched DRB eplets: 142M3, 25HRF, 26KFD, 71QAA, 73AADT, and 96QV. Because

Table 3

Example of antibody reactivity of a post-allograft nephrectomy serum from a patient who had rejected a kidney transplant with a DR15 mismatch

Allele	MFI ^a	No. of eplets	Mismatched eplets
DRB1*010	3252	4	12LKF2,14FEH,25HRL, 96EV
DRB1*010	2683	4	12LKF2,14FEH,25HRL, 96EV
DRB1*010	2460	4	12LKF2,14FEH,25HRL, 96EV
DRB1*030	Self 120	0	"
DRB1*030	206	0	"
DRB1*040	317	0	"
DRB1*040	199	0	"
DRB1*040	225	0	"
DRB1*040	401	0	"
DRB1*040	181	0	"
DRB1*070	175	0	"
DRB1*080	Self 173	0	"
DRB1*090	5057	4	14FEH,26KYH,31QGIY,40HFD
DRB1*090	1754	4	14FEH,26KYH,31QGIY,40HFD
DRB1*100	2474	4	12VKF3,14FEH,23HRL,96QV
DRB1*110	125	0	"
DRB1*120	214	0	"
DRB1*120	150	0	"
DRB1*130	165	0	"
DRB1*130	90	0	"
DRB1*140	109	0	"
DRB1*150	IM 3695	3	142M3,71QAA,96QV
DRB1*150	2281	3	142M3,71QAA,96QV
DRB1*160	2511	2	142M3,96QV
DRB1*160	2239	3	142MS,70LDRA,96QV
DRB3*010	Self 157	0	"
DRB3*020	241	0	"
DRB4*010	550	0	"
DRB4*010	710	0	"
DRB5*010	IM 6099	4	31QDIY,40HFD, 96EV ,108T3
DRB5*020	8384	6	6C,31QGIY,40HFD,71QAA, 96EV ,108T3

Patient type: DRB1*0301, DRB1*0801; DRB3*0101 Immunizing donor: DRB1*1501, DRB5*0101 (Case contributed by Andrew Lobashevsky, Indianapolis Transplant Center).
^aAverage MFI with self-alleles is 150, bold values are considered positive.

of its strong association with DRB1*1501, we assigned DRB5*0101 which has 12 mismatched DRB eplets: 25HRF, 31QDIY, 32IYN, 40HFD, 135S, 73AADT, 74DRAA, 96EV, 98KN, 108T3, and 120N.

Table 3 shows the serum reactions with the Luminex panel and the mismatched eplets on reactive alleles after the alleles with negative reactions (*i.e.*, MFI < 1000) had been recorded in HLA-Matchmaker. The reactive DRB1*1501 of the immunizing donor has three mismatched eplets: 142M3, 71QAA, and 96QV. All three DR1 alleles reacted also but they did not share any eplet with DRB1*1501. The immunizing donor's DRB5*0101 gave also a positive reaction and has four mismatched eplets, including 96EV, which is also expressed by all three DR1 alleles but no other DRB allele. This suggests that the reactivity with DR1 is caused by anti-96EV antibodies elicited by DRB5*0101 and not by DRB1*1501.

This serum reacted with DRB1*1502, DRB1*1601, and DRB1*1602; they share 96QV and 142M3 with the immunizing DRB1*1501. The 142M3 eplet represents the three patches 12PKR, 133L, and 142M in different locations on the molecular surface and shared between all DR15 and DR16 alleles. Serologically monospecific anti-DR2 antibodies appear to recognize one or more of them within 142M3. It should be noted that DRB1*1001 gave a positive reaction and that it shares 96QV with the immunizing DRB1*1501; this suggests the presence of an anti-96QV antibody. The positive reactions of DRB1*0901 and DRB1*0902 reflect the sharing of 31QGIY and 40HFD with the immunizing DRB5*0101, and they suggest the presence of antibodies against one or both eplets. In other words, all positive reactions can be readily explained with antibodies against mismatched eplets of the immunizer, and they do not represent so-called third-party antibodies as a conventional analysis might suggest.

This patient had been exposed to six mismatched eplets on the immunizing DRB1*1501, but no antibodies were detected against three of them: 25HRF, 26KFD, and 73AADT. The immunizing DRB5*0101 had eight mismatched eplets that did not seem to elicit specific antibodies: 25HRF, 32IYN, 135S, 73AADT, 74DRAA, 98KN, and 120N. These findings suggest that these eplets were not immunogenic for this patient, and the antibody responses were restricted to 142M3, 71QAA, and 96QV on DRB1*1501 and 31QDIY, 40HFD, 96EV, and 108T3 on DRB5*0101. Alleles with any of these reactive eplets can be considered as unacceptable mismatches (data not shown).

Because DR1 and DR51 share the unique 96EV eplet, we raised the question of whether sensitization by a DR1 mismatch can induce antibodies that also react with DR51. As an example, Table 4 represents a case contributed by Silvia Chrenova at the Slovak Medical University in Bratislava, Slovakia.

This patient typed as DRB1*0701, DRB1*1401; DRB3*0202, DRB4*0101 and had experienced rejection of a kidney from a deceased donor with a single DRB1*0101 mismatch. In this Luminex assay, the average MFI value with self-alleles was 1212 and we considered MFI > 3000 as positive reactions. The immunizing DRB1*0101 as well as DRB1*0102, DRB1*0103, DRB5*0101, and DRB5*0202 gave strong reactions. All of them share 96EV, and this suggests that this patient had anti-96EV-specific antibodies elicited by the DRB1*0101 mismatch. Although DRB5*0101 and DRB5*0202 have other mismatched eplets, it is unlikely that these eplets reacted with antibody because the patient had not been exposed to them.

This patient had been exposed to seven mismatched eplets of the immunizing DRB1*0101: 12LKF2, 14FEH, 25HRL, 26RL, 71QRA, 73AADT, and 96EV. Five of them remained on reactive alleles. There was no antibody reactivity with 26RL- and 73AADT-carrying alleles. The weakly reactive DRB1*0405 shares 71QRA with DRB1*0101 and this suggests the possibility of an antibody to this eplet. DRB1*0901 and DRB1*1001 reacted with patient serum be-

Table 4

Example of antibody reactivity of a post-allograft nephrectomy serum from a patient who had rejected a kidney transplant with a DR1 mismatch

Allele	MFI ^a	No. of eplets	Mismatched eplets on reactive alleles
DRB1*0101	IM 11942	6	12LKF2,14FEH,25HRL,71QRA,74QRAA, 96EV
DRB1*0102	11167	6	12LKF2,14FEH,25HRL,71QRA,74QRAA, 96EV
DRB1*0103	8023	4	12LKF2,14FEH,25HRL, 96EV
DRB1*0401	1494	0	"
DRB1*0402	872	0	"
DRB1*0405	3341	2	71QRA,74QRAA
DRB1*0701	Self 2090	0	"
DRB1*0801	818	0	"
DRB1*0901	3807	4	14FEH,26KYH,31QGIY,40HFD
DRB1*1001	4032	3	12VKP3,14FEH,25HRL
DRB1*1101	826	0	"
DRB1*1201	928	0	"
DRB1*1301	924	0	"
DRB1*1305	879	0	"
DRB1*1401	Self 806	0	"
DRB1*1501	1656	0	"
DRB1*1502	1852	0	"
DRB1*1601	1140	0	"
DRB1*0301	841	0	"
DRB1*0303	953	0	"
DRB3*0101	989	0	"
DRB3*0202	Self 840	0	"
DRB3*0301	765	0	"
DRB4*0101	Self 1111	0	"
DRB5*0101	6584	4	31QDIY,40HFD, 96EV ,108T3
DRB5*0202	6995	5	6C,31QGIY,40HFD, 96EV ,108T3

Patient type: DRB1*0701, DRB1*1401; DRB3*0202, DRB4*0101. Immunizing donor: DRB1*0101 (Case contributed by Silvia Chrenova, Slovak Medical University, Bratislava, Slovakia).

^aAverage MFI with self-alleles is 1212, bold values are considered positive.

cause of an antibody specific for 14FEH and possibly also an antibody against 25HRL, as these eplets are shared with DRB1*0101.

All three DR1 alleles in the panel were reactive; they also share 12LKF2, which actually represents two unique epitopes in different locations on all DR1 alleles, namely, 12LKF and 31QCIY. At this time, we could not determine whether this patient had generated anti-12LKF2 antibodies. Consecutive absorption/elution studies with informative alleles such DRB1*1001 and DRB5*0101 would be needed to verify these antibodies. Nevertheless, this HLA-Matchmaker analysis provides sufficient information about DRB mismatch acceptability for this patient. Any allele with 96EV, 14FEH, and possibly 71QRA and 25HRL should be avoided.

This study was performed in 19 allonephrectomy case patients with either a single mismatch for DR15 ($n = 9$), DR16 ($n = 2$), or DR1 ($n = 8$). Table 5 shows the mismatched eplets on donor alleles and the donor eplets remaining on reactive alleles. All DRB types are shown as four-digit, high-resolution alleles either identified by molecular typing (indicated by a pound [#] sign) or determined as most likely from the HLA-A, B, DR, DQ types and corresponding common alleles.

Nineteen case patients had a mismatch for 96EV, and for 17 of them (90%) this eplet was on reactive alleles. The high frequency of anti-96EV antibody responses demonstrates a considerable immunogenicity of 96EV regardless of its presentation by DR1 or DR51.

Table 5 shows also what eplets on donor DRB alleles appeared to commonly induce specific antibodies. Although DR15 and DR16 had multiple mismatched eplets, these mismatches induced restricted patterns of antibodies reacting with alleles primarily expressing 142M3 (11 of 11 cases, 100%), 71QAA (100%), and/or 96QV (64%). Donor DR51 alleles had an average of nine mismatched eplets, but the antibody responses seemed primarily restricted to 40HFD (82%), 96EV (82%), and 108T3 (91%). Donor DR1 alleles had an average of eight mismatched eplets but four of them seemed dominant on antibody reactive alleles: 12LKF2 (100%), 14FEH (67%), 25HRL (67%), and 96EV (100%). Although absorption/elution studies with selected alleles are necessary to dissect the various eplet-specific antibodies, these findings demonstrate how immunogenic eplets can be distinguished.

Table 5
Antibody responses by allograft nephrectomy patients to eplets on DR1, DR15, DR16 and DR51 mismatches

Case	Recipient DR alleles	Donor alleles	Mismatched eplets on donor alleles	Mismatched eplets on reactive alleles
ADEL-1	DRB1*0802 [#] ; DRB1*1302 [#] DRB3*0101	DRB1*1501 DRB5*0101	142M3,26KFD,71QAA,96QV,140A2,149Q 31QDIY,32IYN,40HFD,135S,74DRAA, 96EV ,98KN,108T3,120N, 140A2,149Q	142M3,71QAA 31QDIY,40HFD, 96EV ,108T3
ADEL-2	DRB1*0401 [#] DRB4*0101	DRB1*1501 [#] DRB5*0101	142M3,32FYN,47DFR,48FR,71QAA, 96QV,98KN,120S 31QDIY,40HFD,67FR,70FDRA,71DRA, 74DRAA, 96EV ,108T3	142M3,32FYN,71QAA 31QDIY,70FDRA,108T3
AV	DRB1*0801; DRB1*0901 DRB4*0101	DRB1*1501 DRB5*0101	142M3,25HRF,26KFD,47DFR,48FR, 71QAA,73AADT, 96EV 25HRF,31QDIY,73AADT,74DRAA, 96EV , 108T3	142M3,71QAA none
EM	DRB1*0803; DRB1*1303 DRB3*0202	DRB1*1501 DRB5*0101	142MS,26KFD,47DFR,48FR,71QAA, 85VV,96QV 31QDIY,32IYN,40HFD,135S,67FR, 70FDRA,74DRAA, 96EV ,98KN,108T3,120N	142M3,71QAA,96QV 31QDIY,40HFD,74DRAA, 96EV , 108T3
HC	DRB1*0301; DRB1*0404 DRB3*0101; DRB4*0101	DRB1*1501 DRB5*0101	142M3,32FYN,71QAA,96QV 31QDIY,40HFD,67FR,70FDRA,71DRA, 74DRAA, 96EV ,108TE	142M3,71QAA,96QV 31QDIY,40HFD,67FR,70FDRA, 71DRA,74DRAA, 96EV ,108T3
ND11	DRB1*0401; DRB1*0701 DRB4*0101	DRB1*1501 DRB5*0101	142M3,47DFR,48FR,71QAA, 96QV,98KS 31QDIY,49HFD,67FR,70FDRA,71DRA, 74DRAA, 96EV ,108T3	142M3,71QAA 31QDIY,40HFD, 96EV ,108T3
RM 70532482	DRB1*0301 [#] ; DRB1*0801 [#] DRB3*0101	DRB1*1501 [#] DRB5*0101	142MS3,25HRF,26KFD,71QAA, 73AADT,96QV 25HRF,31QDIY,32IYN,40HFD,135S, 73AADT,74DRAA, 96EV ,98KN,108T3,120N	142M3,71QAA,96QV 31QDIY,40HFD, 96EV ,108T3
SA	DRB1*0401 [#] ; DRB1*1301 [#] DRB3*0202 [#] ; DRB4*0101	DRB1*1501 [#] DRB5*0101 [#]	142MS,32FYN,71QAA,96QV 31QDIY,40HFD,67FR,70FDRA,71DRA, 74DRAA, 96EV ,108T3	142M3,71QAA,97QV 31QDIY,40HFD, 96EV ,M108T3
ZS	DRB1*0301 [#] ; DRB1*1101 [#] DRB3*0202 [#]	DRB1*1501 DRB5*0101	142M3,26KFD,71QAA,96QV 31QDIY,32IYN,40HFD,135S, 96EV , 98KN,108T3,120N	142M3,71QAA,96QV 31QDIY,32IYN,40HFD,135S, 96EV , 98KN,108T3
2910	DRB1*0405; DRB1*1101 DRB3*0202; DRB4*0101	DRB1*1601 DRB5*0202	142M3,96QV 6C,31QGIY,40HFD,71QAA,81HA, 96EV , 108T3	142M3,96QV 6C,31QGIY,40HFD,71QAA, 96EV ,108T3
POL-8790	DRB1*0301; DRB1*0802 DRB3*0101	DRB1*1602 DRB5*0202	142M3,25HRF,26KFD,67LR,70LDRA, 73AADT,74DRAA,96QV 6C, 25HRF,31QGIY,32IYN,40HFD, 71QAA,73AADT,81HA, 96EV , 98KN,108T3,120N	142M3,70LDRA,96QV 6C,31QGIY,32IYN,40HFD, 71QAA, 96EV ,98KN,108T3
4765	DRB1*0404; DRB1*0701 DRB4*0101	DRB1*0102	12LKF2,14FEH,25HRL,26RL,81HA, 96EV , 98KS	12LKF2, 96EV
290544	DRB1*0403 [#] ; DRB1*0803 [#] DRB4*0103 [#]	DRB1*0101	12LKF2,14FEH,25HRL,26RL,40EFD,47EYR, 73AADT,74QRAA, 96EV	12LKF2,14FEH,25HRL, 74QRAA 96EV
695723	DRB1*1202 [#] ; DRB1*1401 [#] DRB3*0202 [#] ; DRB3*0301 [#]	DRB1*0101	12LKF2,14FEH,25HRL,32IYN,33LYNQ, 71QRA,74QRAA, 96EV	12LKF2,14FEH,25HRL,32IYN, 96EV
ECU-1	DRB1*1101; DRB1*1303 DRB3*0202	DRB1*0101	12LKF2,14FEH,25HRL,26RL,32IYN, 67LR,71QRA,74QRAA, 96EV	12LKF2,14FEH,25HRL, 32IYN, 96EV
MC05048330	DRB1*0301 [#] ; DRB1*0804 [#] DRB3*0101	DRB1*0101 [#] DRB1*0103 [#]	12LKF2,14FEH,25HRL,26RL, 32IYN,40EFD, 47EYR,67LR,71QRA, 73AADT,74QRAAA, 96EV 12LK2,14FEH,25HRL,26RL,32IYN, 40EFD,47EYR,71DEA,73AADT, 74DEAA, 96EV	12LKF2, 96EV 12LKF2, 96EV
ND4	DRB1*0402; DRB1*1302 DRB3*0301; DRB4*0101	DRB1*0101	12LKF2,14FEH,25HRL,26RL,71QRA, 74QRAA, 96EV	12LKF2,14FEH,25HRL, 96EV
SM	DRB1*0401; DRB1*1401 DRB3*0202; DRB4*0101	DRB1*0101	12LKF2,14FEH,25HRL,26RL,71QRA, 74QRAA, 96EV	12LKF2,14FEH,25HRL, 96EV
VS	DRB1*0701 [#] ; DRB1*1401 [#] DRB3*0202 [#] ; DRB4*0101	DRB1*0101 [#]	12LKF2,14FEH,25HRL,26RL,71QRA, 73AADT,74QRAA, 96EV	12LKF2,14FEH,25HRL,71QRA, 74QRAA, 96EV

[#]Indicates high-resolution typing performed by submitting laboratory.

4. Discussion

HLAMatchmaker represents a theoretical model for HLA epitope structure. This algorithm has proved to be clinically useful in analyzing antibody specificities of sera from sensitized patients and the determination of HLA mismatch acceptability [3,4,7, 11–22].

This report describes how HLAMatchmaker can analyze epitope specificity of antibodies induced by a conventionally defined DR2 mismatch. Almost all DR15 and DR16 mismatches induced antibodies that reacted also with DR1. A conventional interpretation of this reactivity would be that DR1 and DR2 share a distinct epitope. However, this assumption is incorrect, because there is no structurally defined epitope uniquely shared between these antigens. A more likely interpretation is that DR51, which strongly associates with DR2, represents a second mismatch that induced specific antibodies that also react with DR1. Indeed, HLAMatchmaker identifies a unique eplet 96EV shared exclusively between the DR51 and DR1 alleles used in the Luminex panel. Conversely, all eight cases of sensitization by DR1, which lacks DR51, lead to antibodies that react also with DR51, and this can be explained only with the sharing of 96EV.

The 96EV eplet has two polymorphic residues in discontinuous sequence locations 96 and 180, which are approximately 3.0–3.5 Ångströms apart on the molecular surface. This eplet seems equivalent to Terasaki's epitope #1055, recognized by a mouse monoclonal antibody and defined by a glutamine in position 96 [23]. Because 17/19 (90%) DR1 or DR51 mismatches in this study induced 96EV-specific antibodies, it seems apparent that 96EV is very immunogenic. Sensitization against either antigen renders both of them as unacceptable mismatches if a transplant is considered. These data show the importance of DR51 as a mismatch capable of inducing an antibody response. A previous study on class II sensitized patients has also demonstrated frequent antibodies against DR51 [7]. We believe that DR51 as well as DR52 and DR53 should be included as clinically relevant components of DRB compatibility determination.

The determination of 96EV-specific antibodies induced by a DR1 mismatch permits a better assessment of DRB mismatch acceptability of potential donors for retransplantation. Although the patient may not have been exposed to DR15 or DR16, these antigens become unacceptable mismatches because they are strongly associated with the 96EV-carrying DR51: DRB1*1501 with DRB5*0101 and DRB1*1601 with DRB5*0202.

HLAMatchmaker-based antibody analyses are best done if information is available about the immunogenetic relationship between immunizer and antibody responder, *i.e.*, the identification of the array of mismatched epitopes that are potentially immunogenic. Although these cases involve the same DR antigen mismatch DR15, DR16, or DR1, there are considerable differences between their mismatched eplet profiles for different patients, and they depend on the class II phenotype of the antibody producer. Table 5 shows that most antibody responses are restricted against a small number of apparently immunogenic eplets. They include 142M3, 71QAA, and 96QV on DR15 or DR16 mismatches. 142M3 appears equivalent to Terasaki's epitope #1603, defined by alloantibodies and shared between DR15 and DR16. 96QV appears distinct from the structurally related Terasaki's epitope #1033, represented only by a glutamine in position 96 and shared between DR10, DR15, DR16, and DR53. The 96QV eplet includes a valine residue in the polymorphic position 180 on the molecular surface approximately 3.5 Ångströms away from position 96; this eplet is on DR10, DR15, and DR16, but not DR53. We could not identify a Terasaki epitope analogous to 71QAA, which is shared between DR15 and DRB5*02.

The antibody responses to DR51 mismatches were primarily restricted to three eplets, including 96EV, as discussed above. 108T3 reflects the combination of three polymorphic patches uniquely shared among all DR51 alleles and is equivalent to Terasaki's

epitope #1402 [23]. We could not find a Terasaki epitope corresponding to 40HFD on DR9 and DR51.

The antibody responses to DR1 mismatches were primarily restricted to five eplets, including 96EV, as discussed above. 12LKF2 represents two polymorphic patches uniquely shared among all DR1 alleles. This eplet represents the epitope recognized by monospecific anti-DR1 antibodies, which have been widely described. 14FEH is on DR1, DR9, and DR10 and corresponds to Terasaki's #1005 [23]. 25HRL is on DR1 and DR10; these antigens share Terasaki's #1004 [23].

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