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# Why can Sensitization by a 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 auspices of the 15th International Histocompatibility Workshop we are conducting a multilaboratory collaborative project to characterize these epitopes and also 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 done for 19 informative kidney transplant cases contributed by 13 laboratories worldwide. There were 11 cases with a single DR2 mismatch (DR15 or DR16) and 9 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 which has no associated DR51. All of them reacted also with DR51 and this could only be explained with 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).

### Keywords

HLAMatchmaker; HLA; epitope structure; eplet; allograft nephrectomy



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## 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 functional epitopes are represented by patches of surface-exposed non-self amino acid residues surrounded by residues within a radius of about three Ångstroms [2]. These patches are referred to as “eplets” and 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].

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 14$^{th}$ and 15$^{th}$ 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 14$^{th}$ 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 with 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 15$^{th}$ Workshop project on epitope immunogenicity. About 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.

## Materials and Methods

### Patients and Sera

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

### 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 manufacturer's 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 (One Lambda Inc., Canoga Park, CA) 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.

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

The DR,DQ version of the HLAMatchmaker antibody analysis program can be downloaded free of charge from the http://HLAMatchmaker.net website. The analysis can be done in six easy steps. 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 (4-digit) alleles. If unavailable, the

serological level HLA antigens need to be converted to 4-digit types that can be estimated from the most common alleles and their linkage to other alleles in various racial 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, 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 appropriate 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, these reactive eplets can be recorded 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.

## Results

This study addresses the antibody response to a DR2 mismatch. Table 3 shows the results of the HLAMatchmaker 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 DRB1*1501 mismatch which has six mismatched DRB eplets: 142M3, 25HRF, 26KFD, 71QAA, 73AADT and 96QV. Because of its strong association with DRB1*1501 we assigned DRB5*0101 which has twelve 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 HLAMatchmaker. 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 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 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 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.

The patient typed as DRB1*0701, DRB1*1401; DRB3*0202, DRB4*0101 and had rejected 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 due to an antibody specific for 14FEH and possibly also an antibody against 25HRL because 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 made 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 HLAMatchmaker 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 done on nineteen allonephrectomy cases 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 # sign) or determined as most likely from the HLA-A, B, DR, DQ types and corresponding common alleles.

Nineteen cases had a mismatch for 96EV and for seventeen 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.

## Discussion

HLAMatchmaker represents a theoretical model for HLA epitope structure and this algorithm has proven 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. This assumption is, however, 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 which 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 only be explained with the sharing of 96EV.

The 96EV eplet has two polymorphic residues in discontinuous sequence locations 96 and 180, which are about 3.0–3.5 Angstroms 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 about 3.5 Angstroms away from position 96 and this eplet is on DR10, DR15, 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 between all DR51 alleles and is equivalent to Terasaki's epitope #1402 [23]. We could not find a Terasaki epitope that corresponds 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 between 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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**Table 1**

List of 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 Varnavidou | 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 |
| 12LKF2[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,*0401/02/04/05,*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 |
| 96EV | 96E/180V | DRB1*0101/02/03, B5*0101,*0202 |
| 96QV | 96Q/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 |
| 108T3[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 |
| 142M3[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 |

[a] represents 12LKF and 31QCIY

[b] represents 12DKY, 104AR and 108T

[c] represents 12PKR, 133L and 142M

**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. Patient type: DRB1*0301, DRB1*0801; DRB3*0101 Immunizing donor: DRB1*1501, DRB5*0101 (Case contributed by Andrew Lobashevsky, Indianapolis Transplant Center).

| Allele | | MFI* | #Ep | Mismatched Eplets |
|---|---|---|---|---|
| DRB1*0101 | | **3252** | 4 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0102 | | **2683** | 4 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0103 | | **2460** | 4 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0301 | Self | 120 | 0 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0302 | | 206 | 0 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0401 | | 317 | 0 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0402 | | 199 | 0 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0403 | | 225 | 0 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0404 | | 401 | 0 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0405 | | 181 | 0 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0701 | | 175 | 0 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0801 | Self | 173 | 0 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0901 | | **5057** | 4 | 14FEH,26KYH,31QGIY,40HFD |
| DRB1*0902 | | **1754** | 4 | 14FEH,26KYH,31QGIY,40HFD |
| DRB1*1001 | | **2474** | 4 | 12VKF3,14FEH,25HRL,96QV |
| DRB1*1101 | | 125 | 0 | 12VKF3,14FEH,25HRL,96QV |
| DRB1*1201 | | 214 | 0 | 12VKF3,14FEH,25HRL,96QV |
| DRB1*1202 | | 150 | 0 | 12VKF3,14FEH,25HRL,96QV |
| DRB1*1301 | | 165 | 0 | 12VKF3,14FEH,25HRL,96QV |
| DRB1*1303 | | 90 | 0 | 12VKF3,14FEH,25HRL,96QV |
| DRB1*1401 | | 109 | 0 | 12VKF3,14FEH,25HRL,96QV |
| DRB1*1501 | IM | **3695** | 3 | 142M3,71QAA,96QV |
| DRB1*1502 | | **2281** | 3 | 142M3,71QAA,96QV |
| DRB1*1601 | | **2511** | 2 | 142M3,96QV |
| DRB1*1602 | | **2239** | 3 | 142M3,70LDRA,96QV |
| DRB3*0101 | Self | 157 | 0 | 142M3,70LDRA,96QV |
| DRB3*0202 | | 241 | 0 | 142M3,70LDRA,96QV |
| DRB4*0101 | | 550 | 0 | 142M3,70LDRA,96QV |
| DRB4*0103 | | 710 | 0 | 142M3,70LDRA,96QV |
| DRB5*0101 | IM | **6099** | 4 | 31QDIY,40HFD,**96EV**,108T3 |
| DRB5*0202 | | **8384** | 6 | 6C,31QGIY,40HFD,71QAA,**96EV**,108T3 |

*Average MFI with self-alleles is 150, bold values are considered positive.

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

| Allele | | MFI* | #Ep | 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 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0402 | | 872 | 0 | 12LKF2,14FEH,25HRL,**96EV** |
| DRB1*0405 | | **3341** | 2 | 71QRA,74QRAA |
| DRB1*0701 | Self | 2090 | 0 | 71QRA,74QRAA |
| DRB1*0801 | | 818 | 0 | 71QRA,74QRAA |
| DRB1*0901 | | **3807** | 4 | 14FEH,26KYH,31QGIY,40HFD |
| DRB1*1001 | | **4032** | 3 | 12VKF3,14FEH,25HRL |
| DRB1*1101 | | 826 | 0 | 12VKF3,14FEH,25HRL |
| DRB1*1201 | | 928 | 0 | 12VKF3,14FEH,25HRL |
| DRB1*1301 | | 924 | 0 | 12VKF3,14FEH,25HRL |
| DRB1*1303 | | 879 | 0 | 12VKF3,14FEH,25HRL |
| DRB1*1401 | Self | 806 | 0 | 12VKF3,14FEH,25HRL |
| DRB1*1501 | | 1656 | 0 | 12VKF3,14FEH,25HRL |
| DRB1*1502 | | 1852 | 0 | 12VKF3,14FEH,25HRL |
| DRB1*1601 | | 1140 | 0 | 12VKF3,14FEH,25HRL |
| DRB1*0301 | | 841 | 0 | 12VKF3,14FEH,25HRL |
| DRB1*0303 | | 953 | 0 | 12VKF3,14FEH,25HRL |
| DRB3*0101 | | 989 | 0 | 12VKF3,14FEH,25HRL |
| DRB3*0202 | Self | 840 | 0 | 12VKF3,14FEH,25HRL |
| DRB3*0301 | | 765 | 0 | 12VKF3,14FEH,25HRL |
| DRB4*0101 | Self | 1111 | 0 | 12VKF3,14FEH,25HRL |
| DRB5*0101 | | **6584** | 4 | 31QDIY,40HFD,**96EV**,108T3 |
| DRB5*0202 | | **6995** | 5 | 6C,31QGIY,40HFD,**96EV**,108T3 |

* Average MFI with self-alleles is 1212, bold values are considered positive.

**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 | Mismatch |
|---|---|---|---|---|
| ADEL-1 | DRB1*0802#; DRB1*1302# | DRB1*1501 | 142M3,26KFD,71QAA,96QV,140A2,149Q | 142M3,71 |
| | DRB3*0101 | DRB5*0101 | 31QDIY,32IYN,40HFD,135S,74DRAA,**96EV**,98KN,108T3,120N,140A2,149Q | 31QDIY,4 |
| ADEL-2 | DRB1*0401# | DRB1*1501# | 142M3,32FYN,47DFR,48FR,71QAA,96QV,98KS,120S | 142M3,32 |
| | DRB4*0101 | DRB5*0101 | 31QDIY,40HFD,67FR,70FDRA,71DRA,74DRAA,**96EV**,108T3 | 31QDIY,7 |
| AV | DRB1*0801; DRB1*0901 | DRB1*1501 | 142M3,25HRF,26KFD,47DFR,48FR,71QAA,73AADT,96QV | 142M3,71 |
| | DRB4*0101 | DRB5*0101 | 25HRF,31QDIY,73AADT,74DRAA,**96EV**,108T3 | none |
| EM | DRB1*0803; DRB1*1303 | DRB1*1501 | 142M3,26KFD,47DFR,48FR,71QAA,85VV,96QV | 142M3,71 |
| | DRB3*0202 | DRB5*0101 | 31QDIY,32IYN,40HFD,135S,67FR,70FDRA,74DRAA,**96EV**,98KN,108T3,120N | 31QDIY,4 |
| HC | DRB1*0301; DRB1*0404 | DRB1*1501 | 142M3,32FYN,71QAA,96QV | 142M3,71 |
| | DRB3*0101; DRB4*0101 | DRB5*0101 | 31QDIY,40HFD,67FR,70FDRA,71DRA,74DRAA,**96EV**,108T3 | 31QDIY,4 |
| ND11 | DRB1*0401; DRB1*0701 | DRB1*1501 | 142M3,47DFR,48FR,71QAA,96QV,98KS | 142M3,71 |
| | DRB4*0101 | DRB5*0101 | 31QDIY,40HFD,67FR,70FDRA,71DRA,74DRAA,**96EV**,108T3 | 31QDIY,4 |
| RM 70532482 | DRB1*0301#; DRB1*0801# | DRB1*1501# | 142M3,25HRF,26KFD,71QAA,73AADT,96QV | 142M3,71 |
| | DRB3*0101 | DRB5*0101 | 25HRF,31QDIY,32IYN,40HFD,135S,73AADT,74DRAA,**96EV**,98KN,108T3,120N | 31QDIY,4 |
| SA | DRB1*0401#; DRB1*1301# | DRB1*1501# | 142M3,32FYN,71QAA,96QV | 142M3,71 |
| | DRB3*0202#; DRB4*0101 | DRB5*0101# | 31QDIY,40HFD,67FR,70FDRA,71DRA,74DRAA,**96EV**,108T3 | 31QDIY,4 |
| ZS | DRB1*0301#; DRB1*1101# | DRB1*1501 | 142M3,26KFD,71QAA,96QV | 142M3,71 |
| | DRB3*0202# | DRB5*0101 | 31QDIY,32IYN,40HFD,135S,**96EV**,98KN,108T3,120N | 31QDIY,3 |
| 2910 | DRB1*0405; DRB1*1101 | DRB1*1601 | 142M3,96QV | 142M3,96 |
| | DRB3*0202; DRB4*0101 | DRB5*0202 | 6C,31QGIY,40HFD,71QAA,81HA,**96EV**,108T3 | 6C,31QGI |
| POL-8790 | DRB1*0301; DRB1*0802 | DRB1*1602 | 142M3,25HRF,26KFD,67LR,70LDRA,73AADT,74DRAA,96QV | 142M3,70 |
| | DRB3*0101 | DRB5*0202 | 6C,25HRF,31QGIY,32IYN,40HFD,71QAA,73AADT,81HA,**96EV**,98KN,108T3,120N | 6C,31QGI |
| 4765 | DRB1*0404; DRB1*0701 DRB4*0101 | DRB1*0102 | 12LKF2,14FEH,25HRL,26RL,81HA,**96EV**,98KS | 12LKF2,**9** |
| 290544 | DRB1*0403#; DRB1*0803# DRB4*0103# | DRB1*0101 | 12LKF2,14FEH,25HRL,26RL,40EFD,47EYR,73AADT,74QRAA,**96EV** | 12LKF2,1 |
| 695723 | DRB1*1202#; DRB1*1401# DRB3*0202#; DRB3*0301# | DRB1*0101 | 12LKF2,14FEH,25HRL,32IYN,33LYNQ,71QRA,74QRAA,**96EV** | 12LKF2,1 |
| ECU-1 | DRB1*1101; DRB1*1303 DRB3*0202 | DRB1*0101 | 12LKF2,14FEH,25HRL,26RL,32IYN,67LR,71QRA,74QRAA,**96EV** | 12LKF2,1 |
| MC 05048330 | DRB1*0301#; DRB1*0804# | DRB1*0101# | 12LKF2,14FEH,25HRL,26RL,32IYN,40EFD,47EYR,67LR,71QRA,73AADT,74QRAAA,**96EV** | 12LKF2,**9** |
| | DRB3*0101 | DRB1*0103# | 12LKF2,14FEH,25HRL,26RL,32IYN,40EFD,47EYR,71DEA,73AADT,74DEAA,**96EV** | 12LKF2,**9** |
| ND4 | DRB1*0402; DRB1*1302 DRB3*0301; DRB4*0101 | DRB1*0101 | 12LKF2,14FEH,25HRL,26RL,71QRA,74QRAA,**96EV** | 12LKF2,1 |
| SM | DRB1*0401; DRB1*1401 DRB3*0202; DRB4*0101 | DRB1*0101 | 12LKF2,14FEH,25HRL,26RL,71QRA,74QRAA,**96EV** | 12LKF2,1 |
| VS | DRB1*0701#; DRB1*1401# DRB3*0202#; DRB4*0101 | DRB1*0101# | 12LKF2,14FEH,25HRL,26RL,71QRA,73AADT,74QRAA,**96EV** | 12LKF2,1 |

# Indicates high-resolution typing performed by submitting laboratory