

Original Research

## Eplet-Based HLA Class II Matching for Transplantation: Design of a Repertoire of Interlocus Eplets Shared between HLA-DR, -DQ and -DP Alleles

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### Abstract

Many studies have demonstrated that HLA-DR, HLA-DQ and HLA-DP matching at the eplet level reduces allograft rejection and improves transplant outcome. Such studies have examined the eplet effect for the individual class II loci, but until now little attention has been given to so-called interlocus class II eplets shared between HLA-DR, HLA-DQ and/or HLA-DP alleles. This report summarizes current information about antibody-verified interlocus class II eplets. It describes a structural modeling method to determine potentially immunogenic interlocus class II eplets and to identify non-immunogenic eplets because they are monomorphic at another class II locus. We propose that the inclusion of interlocus class II eplets will enhance the efficiency of eplet-based HLA-DR, -DQ, -DP matching in organ transplantation.

### Keywords

HLA matching; eplet; HLA-DRDQDP; interlocus; HLAMatchmaker; HLA antibodies



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## 1. Introduction

There are now many reports that HLA-DR, HLA-DQ and HLA-DP matching at the eplet level is associated with reduced allograft rejection and better outcome of kidney, heart, lung, pancreas and liver transplants [1-21]. These studies have examined the eplet effect for the individual class II loci, but we must raise the question that certain class II eplets are shared between HLA-DR, HLA-DQ and/or HLA-DP alleles, the so-called interlocus class II eplets. This concept is analogous to sharing of antibody-verified class I eplets by combinations of HLA-A, -B and/or -C alleles such as 62GE shared between A2 and B17, 82LR shared between A23, A24, A25, A32 and Bw4-carrying HLA-B alleles and, 163LW shared between various HLA-A, -B and -C alleles. The HLA-ABC eplet repertoire of the International HLA Epitope Registry (<http://www.epregistry.com.br>) has additional antibody-verified and non-verified interlocus class I eplets.

HLA-DR matching at the eplet level considers DRB1 together with DRB3, DRB4 or DRB5 and several antibody-verified DRB eplets are shared between different DRB loci. Examples include 4Q (on DRB1\*07, DRB1\*09 and DRB4\*01), 74R (on DRB1\*03 and DRB3\*01:01) and 96EV (on DRB1\*01, DRB5\*01:01 and DRB5\*02:02). Except for the well-documented, antibody-verified eplet shared between DR11 and a group of DPB alleles (details are below), there is little information about interlocus eplet sharing between DR, DQ and DP. Four-digit allele-based HLA typing for all class II loci is now readily available in histocompatibility laboratories and this permits a determination of the full HLA-DR, -DQ, -DP eplet repertoire.

This report describes our method to characterize interlocus class II eplets. Using aligned amino acid sequences of  $\alpha$  or  $\beta$  chains we have identified a group of interlocus eplets that are defined by one or two polymorphic residues uniquely shared between alleles controlled by combinations of DR, DQ and DP loci. Consistent with our previously reported molecular modelling strategy [22], we defined eplet structures by identifying with the Cn3D program all residues within a 3.5 Ångstrom radius of the polymorphic residue(s).

Besides describing studies presenting preliminary evidence of a few antibody-verified interlocus class II eplets, this report describes a structural modelling method to identify other interlocus eplets on class II  $\beta$  chains and  $\alpha$  chains as potential candidates for experimental verification with informative antibodies.

We also report our findings that certain eplets are defined residues that are polymorphic at one class II locus but are monomorphic at another class II locus. Such eplets may be considered donor-recipient matches and their corresponding interlocus eplets may not be immunogenic.

The inclusion of interlocus class II eplets offers opportunities that will increase the accuracy of HLA-DR, -DQ, -DP matching at the eplet level.

## 2. Methods and Results

### 2.1 Comparisons of Amino Acid Sequences of DR, DQ and DP Chains

The protein BLAST (Basic Local Alignment Search Tool) program [23] is a commonly used tool to determine sequence and structural similarities between different proteins. This program can be accessed at [www.blast.ncbi.nlm](http://www.blast.ncbi.nlm) and upon entering amino acid sequences of two proteins it generates the following information: (1) Statistical significance of the residue alignment between two proteins, (2) Identification of the number and percentage of sequence positions with identical

residues, (3) the number and percentage of sequence positions with identical residues together with non-identical residues with positive BLOSUM scores and (4) the number of gaps in the sequence alignment.

In bioinformatics, BLOSUM (BLOcks SUBstitution Matrix) is used to score alignments between evolutionary divergent protein sequences [24]. This has been done by calculating a log-odds score for each of the 210 possible substitution pairs of the 20 standard amino acids in 2000 aligned blocks of 500 groups of related proteins. For each amino acid substitution between two aligned sequences, BLOSUM has scores as positive (see Table S1), zero or negative. For instance, leucine has positive BLOSUM scores with structurally similar isoleucine (+2), methionine (+2) and valine (+3), a 0 score with phenylalanine and negative scores with the remaining residues such as alanine (-1) and asparagine (-4). The BLOSUM matrix is considered a useful guide to study the evolutionary and chemical relationship between various proteins [25].

A BLAST analysis of HLA-DR, HLA-DQ and HLA-DP proteins has, as expected, a very high degree of sequence homology. As an example, Table S2 shows the results for class II  $\beta$  chains: DRB1\*01:01 versus DQB1\*02:01, DRB1\*01:01 versus DPB1\*01:01 and DQB1\*02:01 versus DPB1\*01:01. In all cases the structural homology was statistically highly significant because the  $p$  values are less than  $1 \times 10^{-90}$ . The numbers of identical residue combinations in the amino acid 1-190 sequence ranged between 127 to 138 (67% to 73%). About 80% of the residues had a positive alignment; they included 22, 24 and 14 non-identical residue pairs with positive BLOSUM scores (indicated by a "+" sign). BLAST analyses with other DRB, DQB and DPB allelic combinations yielded similar results (data not shown). The HLA-DP sequences show gaps in sequence positions 24 and 25 when compared with HLA-DRB and HLA-DQB and there is also a gap in position 154. These gaps in DPB lack amino acid residues present in DRB and DQB.

Table S3 shows the BLAST analysis data for class II  $\alpha$  chains namely, DRA1\*01:01, DQA1\*01:01 and DPA1\*01:03. The frequencies of identical residues ranged from 58% to 63%, somewhat lower than for the  $\beta$  chains; 72% to 74% of the residues had a positive alignment. The sequences of DRA and DPA have gaps in positions 1,2 and 15 when compared with DQA sequences.

## **2.2 Interlocus Eplet Assignments on $\beta$ Chains**

Interlocus eplets were determined for the 1-190 sequences adjusted for residue identity positions on HLA-DRB, -DQB and -DPB chains. To account for the three gaps in the 1-190 sequence of HLA-DPB, adjustments were made whereby DPB positions 24-153 became 26-155 and DPB positions 154-190 became 157-193. Interlocus eplets were assigned by identifying in each sequence location one or more polymorphic residues shared between two or all three class II loci. Interlocus eplets were assigned with prefixes such as "rq", "rp", "qp" or "rqp", indicating the sharing between DRB, DQB and/or DPB alleles. This report addresses only class II alleles that are commonly used in antibody testing assays with single allele beads.

For all eplet-carrying alleles we have determined with the Cn3D molecular modelling program [26] which amino acid residues are located within a 3.5 Ångstrom radius of the residue(s) used to assess the dimension of an eplet [22]. For each interlocus eplet we have identified within that area, the most commonly shared residues with standard one-letter amino acid codes. For each allele, identical residues are displayed with dash symbols and different residues are shown with the one-letter amino acid codes. Residue differences with positive BLOSUM scores (Table S1) as

indicators of a structural and evolutionary relationship have been marked with an asterisk. For instance, the difference between alanine and serine has a +1 BLOSUM score and substitutions for A are marked as S\* and substitutions for S are marked as A\*; other substitutions for these residues are marked with the usual single letter codes without an asterisk.

Interlocus eplets on class II  $\alpha$  chains were also determined for DRA, DQA and DPA. They were based on the 1-190 sequence of DQA alleles and sequences of DRA and DPA were adjusted to DQA after identifying a gap in position 15. Since DRA is largely monomorphic, interlocus eplets can only be between DQA and DPA alleles; they have the “qp” prefix.

### 2.3 Antibody-Verified Interlocus rp58E and rp58EE Eplets

The best documentation of an antibody-verified class II interlocus eplet is shared between DR11 and a group of DPB alleles. Table 1 describes two variations. Human and mouse monoclonal antibodies are specific for rp58E shared between DRB1\*11:01/03/04 and DPB1\*02, \*03, \*04:02, \*06, \*09, \*10, \*14, \*16, \*17, \*18, \*20 and \*28 [27-34]. All rp58E-carrying alleles have the same 54G, 55R, 56P, 57D, 60Y, 61W and 62N residues within a 3.5 Ångstrom radius and the reactivity of a rp58E-specific antibody is unaffected by the presence of 59D\* which has a Blosum score of +2 when compared to 59E. Table 1 shows also a second antibody reactivity pattern defined by rp58EE which is shared between DRB1\*11:01/03/04 and DPB1\*02, \*04:02, \*10, \*16, \*18 and \*28 [33, 35, 36]; this eplet is defined by residues 58E and 59E and it is apparent that for this antibody, a substitution by 59D\* has a negative effect.

**Table 1** Reactivity patterns of antibody-verified rp58E and rp58EE eplets and amino acid residues within a 3.5 Ångstrom radius.

|            | 54 | 55 | 56 | 57 | 58      | 59 | 60 | 61 | 62 | Antibody-verified |        |
|------------|----|----|----|----|---------|----|----|----|----|-------------------|--------|
|            | G  | R  | P  | D  | Residue | E  | Y  | W  | N  | Eplet             | Eplet  |
| DRB1*11:01 | -  | -  | -  | -  | E       | -  | -  | -  | -  | rp58E             | rp58EE |
| DRB1*11:02 | -  | -  | -  | -  | E       | -  | -  | -  | -  | rp58E             | rp58EE |
| DRB1*11:04 | -  | -  | -  | -  | E       | -  | -  | -  | -  | rp58E             | rp58EE |
| DPB1*02:01 | -  | -  | -  | -  | E       | -  | -  | -  | -  | rp58E             | rp58EE |
| DPB1*04:02 | -  | -  | -  | -  | E       | -  | -  | -  | -  | rp58E             | rp58EE |
| DPB1*08:01 | -  | -  | -  | -  | E       | -  | -  | -  | -  | rp58E             | rp58EE |
| DPB1*10:01 | -  | -  | -  | -  | E       | -  | -  | -  | -  | rp58E             | rp58EE |
| DPB1*16:01 | -  | -  | -  | -  | E       | -  | -  | -  | -  | rp58E             | rp58EE |
| DPB1*18:01 | -  | -  | -  | -  | E       | -  | -  | -  | -  | rp58E             | rp58EE |
| DPB1*28:01 | -  | -  | -  | -  | E       | -  | -  | -  | -  | rp58E             | rp58EE |
| DPB1*03:01 | -  | -  | -  | -  | E       | D* | -  | -  | -  | rp58E             |        |
| DPB1*06:01 | -  | -  | -  | -  | E       | D* | -  | -  | -  | rp58E             |        |
| DPB1*09:01 | -  | -  | -  | -  | E       | D* | -  | -  | -  | rp58E             |        |
| DPB1*14:01 | -  | -  | -  | -  | E       | D* | -  | -  | -  | rp58E             |        |
| DPB1*17:01 | -  | -  | -  | -  | E       | D* | -  | -  | -  | rp58E             |        |
| DPB1*20:01 | -  | -  | -  | -  | E       | D* | -  | -  | -  | rp58E             |        |

## 2.4 Predictions of Other Interlocus Class II Eplets in the 54-62 Sequence

This sequence has four additional interlocus eplets with a high degree of amino acid residue similarity. None have been verified experimentally with informative antibodies, but they might be suitable candidates. These eplets are defined by polymorphic residues in position 57 and the interlocus eplets are called rq57S, rqp57A, rq57V and rqp57D (Table 2). DQB1\*05:02 shares rq57S with DRB1\*04:05, \*08:01 and \*13:03 and the residues in the 54-62 sequence are identical. The rqp57A eplet is shared between DRB1\*14:01, DQB1\*02 and \*03:02 and DPB1\*01, \*04:01, \*11, \*13, \*15 and \*23 have considerable 54-62 sequence similarity but it is possible that the difference in position 55, i.e. 55R versus 55L and/or 55P may affect cross-reactivity with antibody. The rq57V eplet shared between DRB1\*07, \*09, \*12, DRB3\*01:01, \*03:01 and DQB1\*05:01, \*06:04/09 have identical residues in positions 54, 55, 56, 58, 59, 61 and 62; the only difference is at position 60 whereby the DRB alleles have 60S and the DQB alleles have 60Y.

**Table 2** Allele sharing for four interlocus eplets in sequence position 57 and amino acid residues within a 3.5 Ångstrom radius.

|            | 54 | 55 | 56 | 57  | 58 | 59 | 60 | 61 | 62 | Eplet  |                  | 54 | 55 | 56 | 57      | 58 | 59 | 60 | 61 | 62 | Eplet |        |
|------------|----|----|----|-----|----|----|----|----|----|--------|------------------|----|----|----|---------|----|----|----|----|----|-------|--------|
|            | G  | R  | P  | sid | A  | E  | Y  | W  | N  |        |                  | G  | R  | P  | Residue | A  | E  | Y  | W  | N  |       |        |
| DRB1*04:05 | -  | -  | -  | 57S | -  | -  | -  | -  | -  | rq57S  | DRB1*01          | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DRB1*08:01 | -  | -  | -  | 57S | -  | -  | -  | -  | -  | rq57S  | DRB1*03          | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DRB1*13:03 | -  | -  | -  | 57S | -  | -  | -  | -  | -  | rq57S  | DRB1*04:01       | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DQB1*05:02 | -  | -  | -  | 57S | -  | -  | -  | -  | -  | rq57S  | DRB1*04:02       | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
|            |    |    |    |     |    |    |    |    |    |        | DRB1*04:03       | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
|            |    |    |    |     |    |    |    |    |    |        | DRB1*04:04       | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DRB1*14:01 | -  | -  | -  | 57A | -  | -  | H* | -  | -  | rqp57A | DRB1*08:02       | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DQB1*02:01 | -  | L  | -  | 57A | -  | -  | -  | -  | -  | rqp57A | DRB1*09:02       | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DQB1*02:02 | -  | L  | -  | 57A | -  | -  | -  | -  | -  | rqp57A | DRB1*10:01       | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DQB1*03:02 | -  | P  | -  | 57A | -  | -  | -  | -  | -  | rqp57A | DRB1*11:01       | -  | -  | -  | 57D     | E  | -  | -  | -  | -  | -     | rqp57D |
| DPB1*01:01 | -  | -  | -  | 57A | -  | -  | -  | -  | -  | rqp57A | DRB1*13:01       | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DPB1*04:01 | -  | -  | -  | 57A | -  | -  | -  | -  | -  | rqp57A | DRB1*13:02       | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DPB1*11:01 | -  | -  | -  | 57A | -  | -  | -  | -  | -  | rqp57A | DRB1*14:02       | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DPB1*13:01 | -  | -  | -  | 57A | -  | -  | -  | -  | -  | rqp57A | DRB1*14:54       | -  | -  | -  | 57D     | -  | -  | H* | -  | -  | -     | rqp57D |
| DPB1*15:01 | -  | -  | -  | 57A | -  | -  | -  | -  | -  | rqp57A | DRB1*15          | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DPB1*23:01 | -  | -  | -  | 57A | -  | -  | -  | -  | -  | rqp57A | DRB1*16          | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
|            |    |    |    |     |    |    |    |    |    |        | DRB3*02:02       | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DRB1*07:01 | -  | -  | -  | 57V | -  | -  | S  | -  | -  | rq57V  | DRB4*            | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DRB1*09:01 | -  | -  | -  | 57V | -  | -  | S  | -  | -  | rq57V  | DRB5*            | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DRB1*12:01 | -  | -  | -  | 57V | -  | -  | S  | -  | -  | rq57V  | DQB1*03:01/03    | -  | P  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DRB1*12:02 | -  | -  | -  | 57V | -  | -  | S  | -  | -  | rq57V  | DQB1*04          | -  | -  | L  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DRB3*01:01 | -  | -  | -  | 57V | -  | -  | S  | -  | -  | rq57V  | DQB1*06:01/02/03 | -  | -  | -  | 57D     | -  | -  | -  | -  | -  | -     | rqp57D |
| DRB3*03:01 | -  | -  | -  | 57V | -  | -  | S  | -  | -  | rq57V  | DPB1*02:01       | -  | -  | -  | 57D     | E  | -  | -  | -  | -  | -     | rqp57D |
| DQB1*05:01 | -  | -  | -  | 57V | -  | -  | -  | -  | -  | rq57V  | DPB1*03:01       | -  | -  | -  | 57D     | E  | D* | -  | -  | -  | -     | rqp57D |
| DQB1*06:04 | -  | -  | -  | 57V | -  | -  | -  | -  | -  | rq57V  | DPB1*04:02       | -  | -  | -  | 57D     | E  | -  | -  | -  | -  | -     | rqp57D |
| DQB1*06:05 | -  | -  | -  | 57V | -  | -  | -  | -  | -  | rq57V  | DPB1*06:01       | -  | -  | -  | 57D     | E  | D* | -  | -  | -  | -     | rqp57D |
| DQB1*06:09 | -  | -  | -  | 57V | -  | -  | -  | -  | -  | rq57V  | DPB1*08:01       | -  | -  | -  | 57D     | E  | -  | -  | -  | -  | -     | rqp57D |
|            |    |    |    |     |    |    |    |    |    |        | DPB1*09:01       | -  | -  | -  | 57D     | E  | D* | -  | -  | -  | -     | rqp57D |
|            |    |    |    |     |    |    |    |    |    |        | DPB1*10:01       | -  | -  | -  | 57D     | E  | -  | -  | -  | -  | -     | rqp57D |
|            |    |    |    |     |    |    |    |    |    |        | DPB1*14:01       | -  | -  | -  | 57D     | E  | D* | -  | -  | -  | -     | rqp57D |
|            |    |    |    |     |    |    |    |    |    |        | DPB1*17:01       | -  | -  | -  | 57D     | E  | D* | -  | -  | -  | -     | rqp57D |
|            |    |    |    |     |    |    |    |    |    |        | DPB1*18:01       | -  | -  | -  | 57D     | E  | -  | -  | -  | -  | -     | rqp57D |
|            |    |    |    |     |    |    |    |    |    |        | DPB1*20:01       | -  | -  | -  | 57D     | E  | D* | -  | -  | -  | -     | rqp57D |
|            |    |    |    |     |    |    |    |    |    |        | DPB1*28:01       | -  | -  | -  | 57D     | E  | -  | -  | -  | -  | -     | rqp57D |

Many DRB alleles share rqp57D, and 7 of 8 additional residues with DQB1\*03:01 and \*03:03 which have 55P instead of 55R, and DQB1\*04 which has 56L instead of 56P. It should be noted that the rqp57-carrying DQB1\*06 alleles have identical residues as the rqp57D-carrying DRB alleles. All rqp57D-carrying DPB alleles have 58E instead of 58A and about half of them have 59D\* instead of 59E. It is possible that the reactivity of rqp57D might be affected by residue differences at DPB and DQB1\*03:01/03 and DQB1\*04 and that the interlocus eplet is shared only between DRB alleles and DQB1\*06:01/02/03 that have identical residues within a 3.5 Ångstrom radius.

Given their interlocus structural similarity, it seems that rq57S, rqp57A, rqp57V and rqp57D might be considered as potential candidates for experimental verification with informative antibodies.

### 2.5 Have Other Interlocus Class II Eplets Been Antibody-Verified?

Current information about antibody-verified interlocus class II eplets is quite limited but a few studies have yielded promising data which must nevertheless be considered preliminary. The experimental evidence for three antibody-verified interlocus class II eplets has been summarized as follows:

#### 2.5.1 Antibody-Verified rq70RK/R on DQ2, DR9, DR10, DRB1\*14:01 and Possibly DR53.

Drover et al [37] described a mouse monoclonal antibody NFLD.M71 that reacted in binding assays using homozygous cell lines with a polymorphic determinant shared between DQB1\*02:01 chains and DRB1\*14:01, DR9 and DR10. From an amino acid sequence analysis, it appears that NFLD.M71 recognizes a structural determinant determined by residues 70-73, RKRA on DQBI\*02:01 chains and RRRRA on DRBI\*09:01/02, \*10:01, and \*14:01 chains. Our analysis suggests the interlocus rq70RK/R eplet defined by residues 70R+71K\*on DQB or 70R+71R\*on DRB (Table 3). Six of seven positions within a 3.5 Ångstrom radius have identical residues. No information was provided about the reactivity of DRB4 alleles which also carry rq70R+71R\*.

**Table 3** Antibody-verified interlocus eplet rq70RK/R and amino acid residues within 3.5 Ångstroms.

| Allele     | NFLD.M71 Antibody | 68 | 69 | 7071 Residues | 72 | 73 | 74 | 75 | 76 | Eplet    |
|------------|-------------------|----|----|---------------|----|----|----|----|----|----------|
|            |                   | L  | E  |               | R  | A  | E  | V  | D  |          |
| DQB1*02:01 | Reactive          | -  | -  | 70R71K*       | -  | -  | A  | -  | -  | rq70RK/R |
| DQB1*02:02 | Not tested?       | -  | -  | 70R71K*       | -  | -  | A  | -  | -  | rq70RK/R |
| DRB1*09:01 | Reactive          | -  | -  | 70R71R*       | -  | -  | -  | -  | -  | rq70RK/R |
| DRB1*09:02 | Reactive          | -  | -  | 70R71R*       | -  | -  | -  | -  | -  | rq70RK/R |
| DRB1*10:01 | Reactive          | -  | -  | 70R71R*       | -  | -  | A  | -  | -  | rq70RK/R |
| DRB1*14:01 | Reactive          | -  | -  | 70R71R*       | -  | -  | -  | -  | -  | rq70RK/R |
| DRB4*01:01 | Not tested?       | -  | -  | 70R71R*       | -  | -  | -  | -  | -  | rq70RK/R |
| DRB4*01:03 | Not tested?       | -  | -  | 70R71R*       | -  | -  | -  | -  | -  | rq70RK/R |

### 2.5.2 Antibody-Verified rp67IE on DRB1\*01:03 and Several DP Alleles

Callender et al [38] reported allosera with strong cross-reactivity between DP and DRB1\*01:03 but not with other DRB1\*01 alleles. Sequence alignments suggested a new cross-reactivity between DRB1\*01:03 and DPB1\*02, \*09, \*10, \*13, \*16 and \*17. Two additional sera confirmed this cross-reactivity, although no data were presented about antibody reactivity patterns with the HLA panel. This epitope was originally described as 65I+67E on DP and 67I+69E on DR but according to the new sequence alignment described above the interlocus eplet can be called rp67IE as defined by residues 67I and 71E.

Eight residues within a 3.5 Ångstrom radius are identical between DRB1\*01:03 and the rp67IE-carrying DPB1 alleles and the difference between 70D and 70E was acceptable for antibody reactivity (Table 4). The original report [38] indicated that the rp67IE-carrying DRB1\*13:01 was weakly reactive; they have a nearby distinct 28D residue below the molecular surface but close enough to 67I and 71E to influence reactivity with this antibody. Table 4 also explains why DRB1\*01:01 and DRB1\*01:02 were non-reactive; these alleles carry a different eplet, 67LR.

**Table 4** Antibody-verified interlocus eplet rp67IE and amino acid residues within 3.5 Ångstroms.

|               | Antibody | 64 | 65 | 66 | 67       | 71 | 68 | 69 | 70 | 72 | 73 | 28 | Eplet  |
|---------------|----------|----|----|----|----------|----|----|----|----|----|----|----|--------|
|               | Reactive | Q  | K  | D  | Residues |    | L  | E  | E  | R  | A  | E  |        |
| DPB1*02:01    | Positive | -  | -  | -  | 67IE     |    | -  | -  | -  | -  | -  | -  | rp67IE |
| DPB1*09:01    | Positive | -  | -  | -  | 67IE     |    | -  | -  | -  | -  | -  | -  | rp67IE |
| DPB1*10:01    | Positive | -  | -  | -  | 67IE     |    | -  | -  | -  | -  | -  | -  | rp67IE |
| DPB1*13:01    | Positive | -  | -  | -  | 67IE     |    | -  | -  | -  | -  | -  | -  | rp67IE |
| DPB1*16:01    | Positive | -  | -  | -  | 67IE     |    | -  | -  | -  | -  | -  | -  | rp67IE |
| DPB1*17:01    | Positive | -  | -  | -  | 67IE     |    | -  | -  | -  | -  | -  | -  | rp67IE |
| DRB1*01:03    | Positive | -  | -  | -  | 67IE     |    | -  | -  | D* | -  | -  | -  | rp67IE |
| DRB1*13:01    | Weak     | -  | -  | -  | 67IE     |    | -  | -  | D* | -  | -  | D* | rp67IE |
| DRB1*01:01    | Negative | -  | -  | -  | 67LR     |    | -  | -  | Q* | -  | -  | -  |        |
| DRB1*01:02    | Negative | -  | -  | -  | 67LR     |    | -  | -  | Q* | -  | -  | -  |        |
| Other DR orDP | Negative |    |    |    |          |    |    |    |    |    |    |    |        |

### 2.5.3 Antibody-Verified rq75VT on Several DRB Alleles and DQ4.

The IgM human monoclonal antibody JOK1H7 originating in Arend Mulder’s Laboratory at Leiden University Medical Center reacts with 75VT-carrying DRB alleles and additionally, with DQ4 heterodimers, which also have 75VT. All other DQ dimers were negative. This antibody originated from a woman who types as DRB1\*03:01, \*13:01, DRB3\*01:01, DQB1\*02:01, \*06:03 and she was HLA sensitized during pregnancy. Although the HLA type of the child is unknown, her husband types as DRB1\*04:02, \*11:01, DRB3\*02:02, DRB4\*01:03, DQB1\*03:02. This monoclonal antibody reacted with husband’s DRB4\*01:03 (MFI=7349) and DRB1\*11:01 (MFI=3664); both have rq75VT.

Table 5 displays the MFI data with the entire DR and DQ panel. None of the DP heterodimers were reactive (data not shown). Positive reactions with DRB alleles were limited to 75VT-carrying DRB alleles except six alleles that have the combination of nearby residues 67I and 70D. This suggests that the epitope defined by 75VT includes residues in sequence positions 67 and 70; the

presence of 67I+70D abolishes the reactivity with this antibody. We noted significantly lower MFI values (3551 versus 11792,  $p < 0.00001$ ) for the seven 75VT-carrying DRB alleles that have 70D together with 67F or 67L. This suggests that the presence of 70D also has some inhibitory effect. In contrast, the four DRB alleles which have 67I+70Q gave similarly high MFI values and the remaining 75VT-carrying DRB alleles also reacted well regardless of residue differences in nearby positions 67 (L and F), 70 (R and Q\*), 71 (R, K\* and A) and 74 (A and E). Other nearby positions 24, 72, 73, 76, 79 and 80 shared the same residue.

**Table 5** Antibody reactivity of rq75VT presented by the immunizing DRB and cross-reactive with DQ4.

|              | MFI    | Eplet  | 24<br>V | 67<br>L | 70<br>R | 71<br>R | 72<br>R | 73<br>A | 74<br>A | 7577<br>Residues | 76<br>D | 78<br>Y | 79<br>C | 80<br>R |
|--------------|--------|--------|---------|---------|---------|---------|---------|---------|---------|------------------|---------|---------|---------|---------|
| DRB1*01:01   | 13223  | rq75VT | -       | -       | Q*      | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*01:02   | 12155  | rq75VT | -       | -       | Q*      | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*04:01   | 12568  | rq75VT | -       | -       | Q*      | K*      | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*04:03   | 11100  | rq75VT | -       | -       | Q*      | -       | -       | -       | E       | 75V77T           | -       | -       | -       | -       |
| DRB1*04:04   | 13067  | rq75VT | -       | -       | Q*      | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*04:05   | 12850  | rq75VT | -       | -       | Q*      | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*09:01   | 12646  | rq75VT | -       | F       | -       | -       | -       | -       | E       | 75V77T           | -       | -       | -       | -       |
| DRB1*09:02   | 13784  | rq75VT | -       | F       | -       | -       | -       | -       | E       | 75V77T           | -       | -       | -       | -       |
| DRB1*10:01   | 13610  | rq75VT | -       | -       | -       | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*14:01   | 13202  | rq75VT | -       | -       | -       | -       | -       | -       | E       | 75V77T           | -       | -       | -       | -       |
| DRB1*15:01   | 11790  | rq75VT | -       | I*      | Q*      | A       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*15:02   | 11544  | rq75VT | -       | I*      | Q*      | A       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*15:03   | 12466  | rq75VT | -       | I*      | Q*      | A       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB4*01:01   | 6010   | rq75VT | -       | -       | -       | -       | -       | -       | E       | 75V77T           | -       | -       | -       | -       |
| DRB4*01:03   | 7349   | rq75VT | -       | -       | -       | -       | -       | -       | E       | 75V77T           | -       | -       | -       | -       |
| DRB5*02:02   | 11303  | rq75VT | -       | I*      | Q*      | A       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*16:01   | 5865   | rq75VT | -       | F       | D       | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*12:02   | 5697   | rq75VT | -       | F       | D       | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*16:02   | 4436   | rq75VT | -       | -       | D       | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*11:01   | 3664   | rq75VT | -       | F       | D       | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*11:04   | 2871   | rq75VT | -       | F       | D       | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*08:01   | 1462   | rq75VT | -       | F       | D       | -       | -       | -       | L       | 75V77T           | -       | -       | -       | -       |
| DRB5*01:01   | 862    | rq75VT | -       | F       | D       | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*13:03   | 402    | rq75VT | -       | I*      | D       | K*      | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*12:01   | 65     | rq75VT | -       | I*      | D       | -       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*01:03   | 19     | rq75VT | -       | I*      | D       | E       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*04:02   | 9      | rq75VT | -       | I*      | D       | E       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*13:01   | 7      | rq75VT | -       | I*      | D       | E       | -       | -       | -       | 75V77T           | -       | -       | -       | -       |
| DRB1*07:01   | 3      | rq75VT | -       | I*      | D       | -       | -       | G       | Q       | 75V77T           | -       | -       | -       | -       |
| DQB1*04:01   | 5471   | rq75VT | -       | I*      | E       | D       | -       | -       | S*      | 75V77T           | -       | V       | -       | -       |
| DQB1*04:02   | 3995   | rq75VT | -       | I*      | E       | D       | -       | -       | S*      | 75V77T           | -       | V       | -       | -       |
| non-75VT DRB | 6±2    |        |         |         |         |         |         |         |         |                  |         |         |         |         |
| non-75VT DQB | 73±194 |        |         |         |         |         |         |         |         |                  |         |         |         |         |

The panel has four heterodimers with DQB1\*04:01 or DQB1\*04:02; all were distinctly reactive with average MFI values of 5471 and 3995, respectively. DQB1\*04 chains carry 75V77T together with 67I+70E. In comparison with the rq75VT-carrying DRB alleles they have identical residues in

positions 24, 72, 73, 76, 79 and 80. On the other hand, residues 70E, 71D, 74S\* and 78V are distinct for DQB1\*04; we cannot determine which residue(s) contribute to the lower MFI values with DQB1\*04 as compared to the high MFI with many DRB alleles.

In conclusion, although this human monoclonal antibody must be considered specific for one epitope, its reactivity pattern with the allele panel was rather complex. The data suggest recognition of a rq75VT-related epitope shared between DRB and DQB.

## 2.6 Predictions of Other Interlocus Class II Eplets on B Chains

This report summarizes the experimental evidence for five antibody-verified interlocus class II eplets; their sequence positions within a 3.5 Ångstrom radius show high degrees of identical residue sharing (see Table 1, Table 3, Table 4 and Table 5). As described above (Table 2), the 54-62 sequence has four interlocus eplets that might be considered as suitable candidates for antibody verification. A search of the entire 1-190 sequence has identified a total of sixteen interlocus class II eplets with comparably similar degrees of residue sharing between eplet-carrying alleles. Table 6 lists these eplets and Table S4 describes the residues within a 3.5 Ångstrom radius. Certain interlocus eplets such as rq26Y, rq37YV and rqp57A are present on relatively small numbers of alleles whereas others are expressed by many alleles encoded by a given locus. Examples are rp37FV on most DPB alleles, rq57D on most DRB alleles and rqp67I on most DRB and DPB alleles. Such interlocus eplets have low probabilities of being mismatched and inducing specific antibodies.

We identified only one α chain interlocus eplet pq34Q shared between DPA1\*02 and all DQA1 alleles except DQA1\*02 (Table S4).

**Table 6** DRB, DQB and DPB alleles with interlocus class II eplets that have not been antibody verified.

| Interlocus Eplet | Luminex Alleles  |
|------------------|--|
| rq26Y            | DRB1*03:01,*09:01,DRB3*01:01, DQB1*03:01,*06:01  |
| rp37FV           | DRB1*07:01,*14:01,*14:54,DRB3*03:01,<br>DPB1*02,*03,*04:02,*06,*08,*09,*10,*14,*16,*17,*18,*19,*20,*23   |
| rqp37YA          | DRB1*10,DRB3*02:02,DRB4, DQB1*03*04,*06:02/03/04/09,<br>DPB1*01,*11,*13,*15  |
| rq37YV           | DRB1*04,*08,*11,*13:03, DQB1*05  |
| rqp57A           | DRB1*14:01, DQB1*02,*03:02, DPB1*01,*04:01,*11,*13,*15,*23   |
| rq57D            | DRB1*01,*03,*04:01/02/03/04,*08:02,*09:02,*10,*11,*13:01/02,*14:02/54,*15,*16,<br>DRB3*02:02,DRB4,DRB5, DQB1*03:01/03,*06:01/02/03<br>DPB1*02,*03,*04:02,*06,*08,*09,*10,*14,*17,*18,*28 |
| rq57S            | DRB1*04:05,*08:01,*13:03, DQB1*05:02   |
| rq57V            | DRB1*07:01,*09:01,*12,DRB3*01:01/03:01, DQB1*05:01,*06:04/09   |
| qp67IE           | DQB1*04, DPB1*01,*02,*04,*05,*08,*09,*10,*13,*17,*18,*19,*23   |
| rqp67IK          | DRB1*13:03, DQB1*02, DPB1*01,*04,*05,*18,*23   |
| rp67LK           | DRB1*03,*04:01, DRB3, DPB1*03,*14,*28  |
| rp67LR           | DRB1*01:01/02,*04:03/05,*10,*14, DRB4*01:01, DPB1*11   |
| rq74AV           | DRB1*01,*04,*10,*11,*12,*13,*14:02,*15,*16, DRB5, DQB1*02  |
| qp77RV           | DQB1*02,*05, DPB1*01,*03,*08,*09,*10,*14   |
| rq77TV           | DRB1*07:01,*09, DQB1*03,*04,*06  |
| rq140TV          | DRB1*03,*04:01,*04:02,*04,*08,*10,*11,*12,*13,*14,DRB3*03:01, DQB1*03,*04  |

## **2.7 Class II Eplets Defined by Polymorphic Residues that Are Monomorphic at Other Loci**

We have also searched the amino acid sequences of class II  $\beta$  and  $\alpha$  chains for eplets that are polymorphic for one locus but monomorphic for another locus. Such eplets might be considered non-immunogenic. This analysis focused on eplets defined by a shared polymorphic residue which is surrounded by at least one identical or structurally very similar residue on each side. Structural comparisons have considered the residues within a 3.5 Ångstrom radius to assess the degree of overall similarity between eplet-carrying alleles. Table S5 shows three DRB eplets (28E, 104S and 133L) and four DQB eplets (30YI, 45GE, 116V and 140A) that are monomorphic at another locus.

Table S6 shows eight DQA eplets (45V, 61G, 66I, 80S, 156F, 160AE, 175E and 175Q) and one DPA eplet (86T) that are monomorphic at another locus. These eplets should be considered self and therefore non-immunogenic because each recipient always has an allele on another locus with the same eplet.

## **3. Discussion**

Although most class II eplets are on alleles controlled by a single HLA-DR, HLA-DQ or HLA-DP locus [39], this report describes our findings about interlocus class II eplets on alleles encoded by combinations of two or three loci. This concept is analogous to the sharing of interlocus class I eplets on combinations of HLA-A, -B and/or -C alleles and their effect on donor-recipient compatibility. So far, interlocus class II eplets have received little attention but with the new abilities of clinical histocompatibility testing laboratories to do high-resolution typing of the entire HLA-DR, -DQ, -DP repertoire there are now opportunities to address this issue.

The inclusion of interlocus class II eplets is important for evaluating donor-recipient compatibility. For example, let us consider the well-documented, antibody-verified rp58E eplet (Table 1). This interlocus eplet is shared between DRB1\*11 and multiple DPB alleles including DPB1\*02, DPB1\*03, DPB1\*04:02 and more. Matching for eplets can be determined by two criteria: acceptability for the sensitized patient and permissibility for the non-sensitized patient. First, consider the sensitized patient who has a specific antibody induced by a rp58E-carrying allele such as DRB1\*11 or DPB1\*02:01; in such case the antibody producer's HLA type must have alleles without rp58E. If a recipient has developed rp58E-specific antibodies induced by DRB1\*11:01 sensitization, then all rp58E-carrying DPB alleles should also be considered unacceptable mismatches although the recipient may have never been exposed to these DPB alleles. This interlocus DPB mismatch unacceptability can be determined only if the potential donor has also been DPB typed.

Conversely, rp58E-specific antibodies can also be induced by a perhaps unknown rp58E-carrying DPB allele; in such case, the reactive DR11 alleles have also become unexpectedly unacceptable mismatches although the recipient has never been exposed to them.

Eplet matching can also assess permissibility for non-sensitized recipients. In the rp58E example, any donor DRB or DPB allele with rp58E must be considered a non-permissible mismatch if all recipient's alleles lack that eplet. Conversely, all other donor alleles without rp58E can be considered permissible mismatches for that eplet.

A complete assessment of class II mismatch acceptability and permissibility should include both locus-specific and interlocus eplets, especially those that have been antibody-verified. The update of the International HLA Epitope Registry (<http://www.epregistry.com.br>) has now considerable

numbers of antibody-verified eplets controlled by the separate HLA-DR, HLA-DQ and HLA-DP loci. The Registry has a new database for interlocus class II eplets that incorporates the information shown in Table 1, Table 2, Table 3, Table 4, Table 5, Table 6 and Table S4. The HLAMatchmaker website ([www.epitopes.com](http://www.epitopes.com)) has been updated with a class II antibody analysis program that includes interlocus eplets.

The question can be raised why the information about antibody-verified interlocus class II eplets is so limited. One might respond that we never looked mainly because antibody producers and immunizers have rarely been typed completely at the DRB1/3/4/5, DQA/B, DPA/B loci at the 4-digit allele level. Although the interlocus class II eplets listed in Table 6 represent theoretical speculations, their experimental verification with informative antibodies has now become feasible in clinical histocompatibility laboratories that require high-resolution DRDQDP typing of antibody producer and immunizer. An excellent source would be human monoclonal antibodies produced by cloned B-cells and tested with single allele panels. Allosera from sensitized patients can also be useful especially if the testing includes absorption-elution studies with informative interlocus-eplet expressing alleles controlled by different loci. Furthermore, mutated alleles with residue substitutions will yield additional information.

Inclusion of interlocus structural comparisons will permit more accurate determinations of class II eplet-based matching important in clinical transplantation. Until now, compatibility has been assessed for separate loci but a better approach would be to consider the DR+DQ+DP repertoire as a single system analogous to matching for HLA-ABC at the eplet level.

### **Additional Materials**

The following additional materials are uploaded at the page of this paper.

1. Table S1: Amino acid residues combinations with positive BLOSUM scores.
2. Table S2: Results of a BLAST analysis between DRB1\*01:01, DQB1\*02:01 and DPB1\*01:01 sequences.
3. Table S3: Allele sharing between interlocus class II eplets and amino acid residues within a 3.5 Ångstrom radius.
4. Table S4: Results of a BLAST analysis between DRA1\*01:01, DQA1\*01:01 and DPA1\*01:03 sequences.
5. Table S5: Amino acid residues within a 3.5 Ångstroms of  $\beta$  chain eplets that are monomorphic at other loci.
6. Table S6: Amino acid residues within a 3.5 Ångstroms of alpha chain eplets that are monomorphic at other loci.

### **Author Contributions**

RJD developed the interlocus concept and together with MM wrote the manuscript

### **Competing Interests**

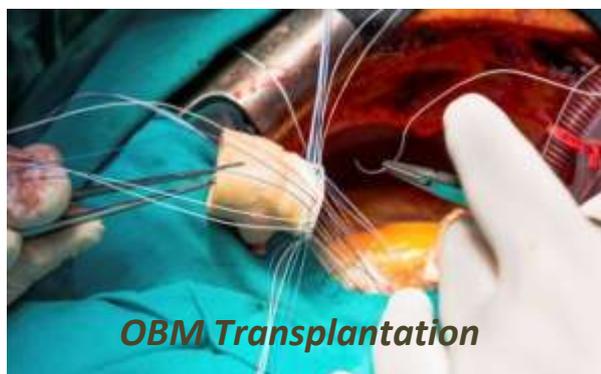
The authors have no conflicts of interest to disclose.

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