Manuscript Details

Manuscript number	HIM_2019_744
Title	Interlocus HLA Class II Cross-reactivity: Design of a Repertoire of Eplets Shared between HLA-DR, -DQ and -DP Alleles
Article type	Research Paper

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	HLAMatchmaker; eplet; class II; HLA-DR,-DQ,-DP; interlocus
Taxonomy	Immunogenetics, Structural Immunology

Corresponding Author	Rene Duquesnoy
Corresponding Author's Institution	UPMC
Order of Authors	Rene Duquesnoy, Marilyn Marrari

Suggested reviewers	Andrew Lobashevsky, Ruth Sapir-Pichhadze, Stefan Schaub

Submission Files Included in this PDF

File Name [File Type]

Cover letter Reg Update interlocus ms.docx [Cover Letter]

DRDQDP interlocus manuscript.docx [Manuscript File]

Tables1-6 Interlocus class II ms.docx [Table]

conflict of interest DRDQDP interlocus manuscript.docx [Conflict of Interest]

Suppl Tables 1-7 Interlocus class II ms.docx [Supplementary Material]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

August 18, 2019

Amy Hahn PhD, Chief Editor Human Immunology

Hi Amy,

We are submitting two manuscripts for your consideration as back to back publications in Human Immunology. The first paper by Duquesnoy, Marrari, da Mata Sousa, Marroquin and do Monte represents an announcement about the HLA Epitope Registry and is entitled “Brief Report: Second update the HLA-DR,-DQ,-DP Eplet Database of the International Registry of HLA Epitopes”.

This paper has a supplemental table about newly antibody-verified class II eplets and another supplemental table showing which eplets have been deleted or have new notations. These findings are being incorporated in an updated version 3.0 of the website-based HLA-DR,-DQ,-DP Registry which will be posted very soon. We welcome any suggestions from the reviewers.

The second manuscript by Duquesnoy and Marrari is entitled “Interlocus HLA Class II Cross-reactivity: Design of a Repertoire of Eplets Shared between HLA-DR, -DQ and -DP Alleles”.

It is now well recognized that eplet-based HLA-DR, HLA-DQ and HLA-DP matching is a superior approach to reduce allograft rejection and improve 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 the limited information currently available 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. This paper is the result of many months of study and analysis. The information in the six tables plus seven supplemental tables will be incorporated in the Registry update after we have received the comments and suggestions from the reviewers.

We believe that the inclusion of interlocus class II eplets will have important implications for eplet-based HLA-DR,-DQ,-DP matching in organ transplantation.

Thank you for considering this pair of papers.

Best regards,

Rene Duquesnoy

August 20, 2019 draft

Interlocus HLA Class II Cross-reactivity: Design of a Repertoire of Eplets Shared between HLA-DR, -DQ and -DP Alleles

Rene. J. Duquesnoy and Marilyn Marrari

Thomas E. Starzl Transplantation Institute
University of Pittsburgh Medical Center, Pittsburgh, PA USA

Summary

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.

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 α or β 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 β chains and α chains as potential candidates for experimental verification with informative antibodies.

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

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

Methods and Results

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 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 Supplemental Table 1), 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 evolutional 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, Supplemental Table 2 shows the results for class II β 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 $1x10^{-90}$. 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.

Supplemental Table 3 shows the BLAST analysis data for class II α 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 β chains; 72% to74% 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.

Interlocus eplet assignments on β chains

Interlocus eplets were determined for the 1-190 sequences adjusted for identical residue 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 (Supplemental Table 1) as indicators of a structural and evolutional 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 α 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 gaps in positions 1, 2 and 15. Since DRA is largely monomorphic, interlocus eplets can only be between DQA and DPA alleles; they have the "qp" prefix.

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 rp58Ecarrying alleles have the same 54G, 55R, 56P, 57D, 60Y, 61W and 62N residues within a 3.5 Ångstrom radius whereas its antibody reactivity is unaffected by the difference between 59E and 59D* which has a Blosum score of +2. 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 59D* has a negative effect.

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.

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, rq57V and rqp57D might be considered as potential candidates for experimental verification with informative antibodies.

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:

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 RRRA 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*

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 DR1 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.

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 means that the presence of 70D has also 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.

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, this human monoclonal antibody which must be specific for one epitope. Although it showed a rather complex reactivity pattern with the allele panel, the data suggest recognition of a rq75VT-related epitope shared between DRB and DQB.

Predictions of other interlocus class II eplets on β 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 Tables 1, 3, 4 and 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 Supplemental Table 4 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 interlocus eplet pq34Q shared between DPA1*02 and all DQA1 alleles except DQA1*02 (Supplemental Table 4).

Class II eplets defined by polymorphic residues that are monomorphic at other loci

We have also searched the amino acid sequences of class II β and α 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. Supplemental table 5 shows three DRB eplets (28E, 104S and 133L) and four DQB eplets (30YI, 45GE, 116V and 140A) that are monomorphic at another locus.

Supplemental Table 6 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 has always an allele on another locus with the same eplet.

Residue analysis of class II eplets with subscript notations

The HLA Epitope Registry has class II eplets annotated with subscripted numbers indicating the presence of several unique residues on separate molecular locations on eplet-carrying alleles. This suggests that these alleles may have two or more eplets that can be recognized by antibody, but current allele panels cannot distinguish between them. For instance, the $45GE_3$-carrying DQB1*02:01 and DQB1*02:02 have unique DQB residues 28S, 30S, 37I, 46E, 47F, 52L, 55L, 71K and 74A. These residues may determine at least three different eplets (hence the subscript 3) but DQ2-specific antibodies cannot distinguish which one(s) are recognized. The interlocus class II eplet concept has been applied to determine which residue is only on DQB1*02 or is shared with DRB and/or DPB alleles. Residue 71K is polymorphic on DRB1 and DPB1 and this residue determines the interlocus eplet rqp67IK whereas 74A is polymorphic for DRB1 and defines the rq74AV eplet (see supplemental Table 4). This suggests that 71K and 74A may not participate in eplets restricted to DQ2 but reflect interlocus eplets.

Residues 46E and 47F are monomorphic on DRB and DPB and they should not be considered as mismatches. This suggests that their corresponding eplet cannot be immunogenic for humans. Interestingly, two mouse monoclonal antibodies have been reported to react specifically with an epitope unique on DQ2 and monomorphic on all DRB and DPB alleles (39, 40). The epitope corresponds to the 45GE eplet which includes 46E and 47F. Accordingly, 45GE may represent a xeno-epitope that can induce antibodies in mice but cannot be immunogenic as an allo-epitope because in humans 45GE is self on all DRB and DPB alleles.

The remaining polymorphic residues 28S, 30S, 37I, 52L and 55L define eplets unique to DQ2. While the HLA Epitope Registry had originally assigned the DQ2-specific eplet as $45GE_3$, it has been reassigned as 52LL in the update.

We have done a similar analysis of other class II eplets with subscripted numbers. Supplemental Table 7 shows which residues are monomorphic on other loci suggesting that they cannot be solely recognized as mismatches that can induce alloantibodies and those residues shared with certain alleles controlled by other loci. Such shared residues might reflect distinct interlocus class II eplets. The remaining residues are locus-specific; they determine eplets on alleles on controlled by a single locus, either DQB, DQA or DRB. For the latter group of residues Supplemental Table 7 shows also the ElliPro scores as described elsewhere (41), and represent estimates in determining eplet immunogenicity. ElliPro values >0.400 suggest increased immunogenicity.

Besides removing the subscripted numbers from locus-specific eplets, these findings necessitated some name changes to better reflect the roles of unique residues in such eplets. As noted above, $45GE_3$ was changed to 52LL. Other examples are the DQA*05-associated eplet $71A_2$ which became 116L because the 71A residue is shared with DRB1*15:01 and DRB5*02:02, and $140A_2$ on DQ2, 5, 6 which became 182S because 140A is monomorphic on DPB.

Discussion

Although most class II eplets are on alleles controlled by a single HLA-DR, HLA-DQ or HLA-DP locus (42), 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 2). 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 has now considerable numbers of antibody-verified eplets controlled by the separate HLA-DR, HLA-DQ and HLA-DP loci (42). The updated Registry also includes a new database with antibody-verified and predicted interlocus class II eplets. It has the information shown in Tables 1-6 and Supplemental Table 4. The HLAMatchmaker website (www.epitopes.com) is being updated with a class II antibody analysis program that incorporates 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 predictions, their experimental verification with informative antibodies has now become feasible in clinical histocompatibility laboratories. Such studies 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.

Conflict of interest statement

The authors have no conflicts of interest to disclose as described by Human Immunology

References

1. Thaunat O, Hanf W, Dubois V, McGregor B, Perrat G, Chauvet C, Touraine J-L, Morelon E. Chronic humoral rejection mediated by anti-HLA-DP alloantibodies: insights into the role of epitope sharing in donor-specific and non-donor specific alloantibodies generation. Transplant immunology. 2009;20:209-211.

2. Singh P, Colombe BW, Francos GC, Martinez Cantarin MP, Frank AM. Acute humoral rejection in a zero mismatch deceased donor renal transplant due to an antibody to an HLA-DP alpha. . Transplantation. 2010;90:220-221.
3. Wiebe C, Nickerson P. Acceptable mismatching at the class II epitope level: the Canadian experience. Current opinion in organ transplantation. 2014;19:442–446.
4. Mierzejewska B, Schroder PM, Baum CE, Blair A, Smith C, Duquesnoy RJ, Marrari M, Gohara A, Malhotra D, Kaw D, Liwski R, Rees MA, Stepkowski S. Early acute antibody-mediated rejection of a negative flow crossmatch 3rd kidney transplant with exclusive disparity at HLA-DP. Hum Immunol. 2014;75:703-708.
5. Wiebe C, Nevins TE, Robiner WN, Thomas W, Matas AJ, Nickerson PW. The Synergistic Effect of Class II HLA Epitope-Mismatch and Nonadherence on Acute Rejection and Graft Survival. Am J Transplant. 2015;15:2197-2202.
6. Sapir-Pichhadze R, Tinckam K, Quach K, Logan AG, Laupacis A, John R, Beyene J, Kim SJ. HLA-DR and -DQ eplet mismatches and transplant glomerulopathy: a nested case-control study. Am J Transplant. 2015;15:137-148.
7. Filippone EJ, Farber JL. Humoral immunity in renal transplantation: epitopes, Cw and DP, and complement activating capability – an update. Clinical transplantation. 2015;29:279-287.
8. Chin N, Westall G, Paraskeva M, Ciciulla J, Cantwell L, Snell G. Challenges inherent to the diagnosis of antibody-mediated rejection in lung transplantation. Respirology case reports. 2015;3:36-39.
9. Bryan CF, Chadha V, Warady BA. Donor selection in pediatric kidney transplantation using DR and DQ eplet mismatching: A new histocompatibility paradigm. Pediatric transplantation. 2016.
10. Walton DC, Hiho SJ, Cantwell LS, Diviney MB, Wright ST, Snell GI, Paraskeva MA, Westall GP. HLA Matching at the Eplet Level Protects Against Chronic Lung Allograft Dysfunction. Am J Transplant. 2016;16:2695-2703.
11. Singh P, Filippone EJ, Colombe BW, Shah AP, Zhan T, Harach M, Gorn C, Frank AM. Sensitization trends after renal allograft failure: the role of DQ eplet mismatches in becoming highly sensitized. Clinical transplantation. 2016;30:71-80.
12. Pouliquen E, Baltzinger P, Lemle A, Chen CC, Parissiadis A, Borot S, Frimat L, Girerd S, Berney T, Lablanche S, Benhamou PY, Morelon E, Badet L, Dubois V, Kessler L, Thaunat O. Anti-Donor HLA Antibody Response After Pancreatic Islet Grafting: Characteristics, Risk Factors, and Impact on Graft Function. Am J Transplant. 2017;17:462-473.
13. Iwami D, Hotta K, Sasaki H, Hirose T, Higuchi H, Takada Y, Shinohara N. Highly Immunogenic DQB1 Mismatch Eplets Are Associated With Development of Chronic Active Antibody-Mediated Rejection: A First Report From Japan. Transplant Proc 2017;49:84-87.
14. Wiebe C, Pochinco D, Blydt-Hansen TD, Ho J, Birk PE, Karpinski M, Goldberg A, Storsley LJ, Gibson IW, Rush DN, Nickerson PW. Class II HLA epitope matching-A strategy to minimize de novo donor-specific antibody development and improve outcomes. Am J Transplant. 2013;13:3114-3122.
15. Kishikawa H, Kinoshita T, Hashimoto M, Fukae S, Taniguchi A, Yamanaka K, Nakagawa M, Nishimura K. Class II HLA Eplet Mismatch Is a Risk Factor for De Novo Donor-Specific Antibody Development and Antibody-mediated Rejection in Kidney Transplantation Recipients. Transplant Proc 2018:2388-2391.
16. Wiebe C, .Kosmoliaptsis V, Pochinco D, Gibson IW, Ho J, Birk PE, Goldberg A, Karpinski M, Shaw J, Rush DN, Nickerson PW. a HLA-DR/DQ Molecular Mismatch: A prognostic biomarker for primary alloimmunity. Am J Transplant. 2018;Nov 10. doi: 10.1111/ajt.15177.
17. Wiebe C, Kosmoliaptsis V, Pochinco D, Taylor C, Nickerson P. A Comparison of HLA Molecular Mismatch Methods to Determine HLA Immunogenicity. Transplantation. 2018;Volume Online First Issue
18. Walton DC, Cantwell L, Hiho S, Ta J, Wright S, Sullivan LC, Snell GI, Westall GP. HLA class II Eplet mismatch predicts De Novo DSA formation post lung transplant. Transpl Immunol 2018;51:73-75.

19. Sypek M, Kausman J, Holt S, Hughes P. HLA Epitope Matching in Kidney Transplantation: An Overview for the General Nephrologist. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2018;71:720-731.
20. Willicombe M, Blow M, Santos-Nunez E, Freeman C, Brookes P, Taube D. Terasaki Epitope Mismatch Burden Predicts the Development of De Novo DQ Donor-Specific Antibodies and are Associated With Adverse Allograft Outcomes. Transplantation. 2018;102:127-134.
21. Ekong UD, Antala S, Bow L, Sese D, Morotti R, Rodriguez-Davalos M, Gan G, Deng Y, Emre SH. HLA, Non-HLA Antibodies, and Eplet Mismatches in Pediatric Liver Transplantation: Observations From a Small, Single-Center Cohort. Experimental and clinical transplantation : official journal of the Middle East Society for Organ Transplantation. 2019;17:6-17.
22. Duquesnoy RJ, Askar M. HLAMatchmaker: A Molecularly Based Algorithm for Histocompatibility Determination V. Eplet Matching for HLA-DR, HLA-DQ and HLA-DP. Human Immunol. 2007;68:12-25.
23. Altschul S, Madden T, Schäffer A, Zhang J, Zhang Z, Miller W, Lipman D. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389-3402.
24. Henikoff S, Henikoff JG. Amino acid substitution matrices from protein blocks. . Proc Natl Acad Sci USA 10915-10919. 1992;89:10915-10919.
25. Thorne JL, Goldman N, Jones DT. Combination of protein evolution and secondary structure. . Mol Biol Evolution 1996;13:666-673.
26. Hogue C. Cn3D: a new generation of three-dimensional molecular structure viewer. Trends Biochem Sci 1997;22:314-316.
27. Bodmer J, Bodmer W, Heyes J, So A, Tonks S, Trowsdale J, Young J. Identification of HLA-DP polymorphism with DP alpha and DP beta probes and monoclonal antibodies: correlation with primed lymphocyte typing. Proceedings of the National Academy of Sciences of the United States of America. 1987;84:4596-4600.
28. Pistillo MP, Mazzoleni O, Kun L, Falco M, Tazzari PL, Ferrara GB. Production of two human hybridomas secreting antibodies to HLA-DRw11 and--DRw8+w12 specificities. Human Immunology. 1991;31:86-93.
29. Yu WY, Watts R, Karr RW. Identification of amino acids in HLA-DPw4β and -DR5β1 chains that are involved in antibody binding epitopes using site-directed mutagenesis and DNA-mediated gene transfer. Human Immunology. 1990;27:122-135.
30. Klohe E, Pistillo MP, Ferrara GB, Goeken NE, Greazel NS, Karr RW. Critical role of HLA-DR beta 1 residue 58 in multiple polymorphic epitopes recognized by xenogeneic and allogeneic antibodies. Human Immunology. 1992;35:18-28.
31. Diaz G, Amicosante M, Jaraquemada D, Butler RH, Gullen MV, Sanchez M, Nombela C, Arroyo J. Functional analysis of HLA-DP polymorphism: a crucial role for DPB residues 9, 11, 35, 55, 56, 69 and 84-87 in T cell allorecognition and peptide binding. International Immunology. 2003;15:565-576.
32. Vaidya S, Hilson B, Sheldon S, Cano P, Fernandez-Vina M. DP reactive antibody in a zero mismatch renal transplant pair. Human Immunology. 2007;68:947-949.
33. Marshall WH, Drover S, Codner D, Gamberg J, Copp MD, Liu HW, Deng LT, Younghusband HB. HLA-DP epitope typing using monoclonal antibodies. Human Immunology. 1998;59:189-197.
34. Cano P, Fernandez-Vina M. Two sequence dimorphisms of DPB1 define the immunodominant serologic epitopes of HLA-DP. Human Immunology. 2009;70:836-843.
35. Tongio MM, Van der Berg Loonen E, Bignon JD, Chandanayingyong D, Dormoy A, Eiermann T, Marshall W, Park MS, Schreuder GMT: HLA-DP detected by serology. in Genetic diversity of HLA Functional and Medical Implication: Proceedings of the Twelfth International Histocompatibility Workshop and Conference. Edited by Charron D. Paris, France, EDK; 1997. pp. 135-138.
36. Inoko H, Bodmer JG, Heyes JM, Drover S, Trowsdale J, Marshall WH: Joint report on the transfectant/monoclonal antibody component. in HLA 1991 Proceedings of the Eleventh International

Histocompatibility Workshop and Conference. Edited by Tsuji K, Aizawa M, Sasazuki T. Oxford, UK, Oxford University Press; 1991. pp. 919-930.
37. Drover S, Gamberg J, Qi C, Marshall WH. Identification of a shared determinant on DQB1*0201 and DRB1*09, DRB1*1001 and DRB1*1401 monoclonal antibody. Hum Immunol. 1992 34:11.
38. Callender CJ, Fernandez-Vina M, Leffell MS, Zachary AA. Frequency of HLA-DP-specific antibodies and a possible new cross-reacting group. Human Immunology. 2012;73:175-179.
39. Ghaderi AA, Gaudernack G, Stanworth DR. Cross-linking of a sequential epitope within the beta-chain of HLA-DR/DP molecules suppressing B lymphocyte growth and inducing homotypic cell aggregation. Immunology Letters. 1994;39:113-119.
40. Congy-Jolivet N, Drocourt D, Portet S, Tiraby G, Blancher A. Production and characterization of chimeric anti-HLA monoclonal antibodies targeting public epitopes as tools for standardizations of the anti-HLA antibody detection. Journal of Immunological Methods. 2013;390:41-51.
41. Duquesnoy RJ, Marrari M. Usefulness of the ElliPro Epitope Predictor Program in Defining the Repertoire of HLA-ABC Eplets. Human Immunology. 2017;74:481-488.
42. Duquesnoy RJ, Marrari M, da Mata Sousa LCD, Marroquim M, do Monte SJH. Brief Report: Second update the HLA-DR,-DQ,-DP eplet database of the International Registry of HLA Epitopes Hum Immunol. 2019;Submitted.

Tables for Interlocus class II eplet ms

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	

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	
	G	R	P	Residue	A	E	Y	W	N	Eplet
DRB1*04:05	-	-	-	57S	-	-	-	-	-	rq57S
DRB1*08:01	-	-	-	57S	-	-	-	-	-	rq57S
DRB1*13:03	-	-	-	57S	-	-	-	-	-	rq57S
DQB1*05:02	-	-	-	57S	-	-	-	-	-	rq57S
DRB1*14:01	-	-	-	57A	-	-	H*	-	-	rqp57A
DQB1*02:01	-	L	-	57A	-	-	-	-	-	rqp57A
DQB1*02:02	-	L	-	57A	-	-	-	-	-	rqp57A
DQB1*03:02	-	P	-	57A	-	-	-	-	-	rqp57A
DPB1*01:01	-	-	-	57A	-	-	-	-	-	rqp57A
DPB1*04:01	-	-	-	57A	-	-	-	-	-	rqp57A
DPB1*11:01	-	-	-	57A	-	-	-	-	-	rqp57A
DPB1*13:01	-	-	-	57A	-	-	-	-	-	rqp57A
DPB1*15:01	-	-	-	57A	-	-	-	-	-	rqp57A
DPB1*23:01	-	-	-	57A	-	-	-	-	-	rqp57A
DRB1*07:01	-	-	-	57V	-	-	S	-	-	rq57V
DRB1*09:01	-	-	-	57V	-	-	S	-	-	rq57V
DRB1*12:01	-	-	-	57V	-	-	S	-	-	rq57V
DRB1*12:02	-	-	-	57V	-	-	S	-	-	rq57V
DRB3*01:01	-	-	-	57V	-	-	S	-	-	rq57V
DRB3*03:01	-	-	-	57V	-	-	S	-	-	rq57V
DQB1*05:01	-	-	-	57V	-	-	-	-	-	rq57V
DQB1*06:04	-	-	-	57V	-	-	-	-	-	rq57V
DQB1*06:05	-	-	-	57V	-	-	-	-	-	rq57V
DQB1*06:09	-	-	-	57V	-	-	-	-	-	rq57V

	54	55	56	57	58	59	60	61	62	
	G	R	P	Residue	A	E	Y	W	N	Eplet
DRB1*01	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*03	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*04:01	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*04:02	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*04:03	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*04:04	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*08:02	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*09:02	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*10:01	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*11:01	-	-	-	57D	E	-	-	-	-	rqp57D
DRB1*13:01	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*13:02	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*14:02	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*14:54	-	-	-	57D	-	-	H*	-	-	rqp57D
DRB1*15	-	-	-	57D	-	-	-	-	-	rqp57D
DRB1*16	-	-	-	57D	-	-	-	-	-	rqp57D
DRB3*02:02	-	-	-	57D	-	-	-	-	-	rqp57D
DRB4*	-	-	-	57D	-	-	-	-	-	rqp57D
DRB5*	-	-	-	57D	-	-	-	-	-	rqp57D
DQB1*03:01/03	-	P	-	57D	-	-	-	-	-	rqp57D
DQB1*04	-	-	L	57D	-	-	-	-	-	rqp57D
DQB1*06:01/02/03	-	-	-	57D	-	-	-	-	-	rqp57D
DPB1*02:01	-	-	-	57D	E	-	-	-	-	rqp57D
DPB1*03:01	-	-	-	57D	E	D*	-	-	-	rqp57D
DPB1*04:02	-	-	-	57D	E	-	-	-	-	rqp57D
DPB1*06:01	-	-	-	57D	E	D*	-	-	-	rqp57D
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*28:01	-	-	-	57D	E	-	-	-	-	rqp57D

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

Allele	NFLD.M71 Antibody	68 L	69 E	7071 Residues	72 R	73 A	74 E	75 V	76 D	Eplet
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

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

	Antibody Reactive	64 Q	65 K	66 D	67 71 Residues	68 L	69 E	70 E	72 R	73 A	28 E	Eplet
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											

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

	MFI	Eplet	24 V	67 L	70 R	71 R	72 R	73 A	74 A	7577 Residues	76 D	78 Y	79 C	80 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													

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, 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, 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
rqp57D	DRB1*01,*03,*04:01/02/03/04,*08:02,*09:02,*10,*11,*13:01/02,*14:02/54,*15,*16, DRB3*02:02,DRB4,DRB5, DQB1*03:01/03,*06:01/02/03 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
rq67LK	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

Interlocus HLA Class II Cross-reactivity: Design of a Repertoire of Eplets Shared between HLA-DR, -DQ and -DP Alleles

Rene. J. Duquesnoy and Marilyn Marrari

Thomas E. Starzl Transplantation Institute
University of Pittsburgh Medical Center, Pittsburgh, PA USA

Conflict of interest statement

The authors have no conflicts of interest to disclose as described by Human Immunology

Supplemental Tables interlocus class II eplet manuscript

Supplemental Table 1 Amino acid residues combinations with positive BLOSUM scores

Residues	BLOSUM	Residues	BLOSUM
A-S	+1	I-M	+1
D-E	+2	I-V	+3
D-N	+1	K-Q	+1
D-T	+1	K-R	+2
E-K	+1	L-M	+2
E-Q	+2	L-V	+1
F-W	+1	M-V	+1
F-Y	+3	N-S	+1
G-T	+1	P-T	+1
H-N	+1	Q-R	+1
H-Y	+2	S-T	+1
I-L	+2	W-Y	+2

Supplemental Table 2 Results of a BLAST analysis between DRB1*01:01, DQB1*02:01 and DPB1*01:01 sequences

```
DRB1*01:01 vs DQB1*02:01 p=3e-100 Identities: 130/190 (68%) Positives: 152/190 (80%) Gaps:0/190
1   GDTRPRFLWQLKFECHFFNGTERVRLLERCIYNQEESVRFDSDVGEYRAVTDLGRPDAEYWNSQ
      D+    F++Q K  C+F NGTERVRL+ R IYN+EE VRFDSDVGE+RAVT LG P AEYWNSQ
1   RDSPEDFVYQFKGMCYFTNGTERVRLVSRSIYNREEIVRFDSDVGEFRAVTLLGLPAAEYWNSQ

65  KDLLEQRRAAVDTYCRHNYGVGESFTVQRRVEPKVTVYPSKTQPLQHHNLLVCSVSGFYPGSI
    KD+LE++RAAVD  CRHNY +     T+QRRVEP VT+ PS+T+ L HHNLLVCSV+ FYP  I
65  KDILERKRAAVDRVCRHNYQLELRTTLQRRVEPTVTISPSRTEALNHHNLLVCSVTDFYPAQI

128 EVRWFRNGQEEKAGVVSTGLI+NGDWTFQTLVMLETVPRSGEVYTCQVEHPSVTSPLTVEWRA
    +VRWFRN QEE AGVVST LI NGDWTFQ LVMLE  P+ G+VYTC VEHPS+ SP+TVEWRA
128 KVRWFRNDQEETAGVVSTPLIRNGDWTFQILVMLEMTPQRGDVYTCHVEHPSLQSPITVEWRA

DRB1*01:01 vs DPB1*01:01 p=8e-95 Identities: 127/190 (67%) Positives: 151/190 (79%) Gaps:3/190
1   GDTRPRFLWQLKFECHFFNGTERVRLLERCIYNQEESVRFDSDVGEYRAVTDLGRPDAEYWNSQKD
      T   ++++Q  +EC  FNGT+R   LER IYN+EE  RFDSDVGE+RAVT LGRP AEYWNSQKD
1   RATPENYVYQGRQECYAFNGTQRxxFLERYIYNREEYARFDSDVGEFRAVTELGRPAAEYWNSQKD

67  LLEQRRAAVDTYCRHNYGVGESFTVQRRVEPKVTVYPSKTQPLQHHNLLVCSVSGFYPGSIEV
    +LE++RA  D  CRHNY + E+ T+QRRV+PKV V PSK  PLQHHNLLVC V+ FYPGSI+V
65  ILEEKRAVPDRVCRHNYEL+EAVTLQRRVQPKVNVSPSKKGPLQHHNLLVCHVTDFYPGSIQV

130 RWFRNGQEEKAGVVSTGLIQNGDWTFQTLVMLETVPRSGEVYTCQVEHPSVTSPLTVEWRA
    RWF NGQEE AGVVST LI+NGDWTF  LVMLE  P+ G+VY CQVEH S+ SP+TVEW A
128 RWFLNGQEETAGVVSTNLIRNGDWTFxILVMLEMTPQQGDVYICQVEHTSLDSPVTVEWKA

DQB1*02:01 vs DPB1*01:01 p=8e-103 Identities: 138/190 (73%) Positives: 152/190 (80%) Gaps:3/190
1   RDSPEDFVYQFKGMCYFTNGTERVRLVSRSIYNREEIVRFDSDVGEFRAVTLLGLPAAEYWNSQ
    R +PE++VYQ +  CY  NGT+R   + R IYNREE  RFDSDVGEFRAVT LG PAAEYWNSQ
1   RATPENYVYQGRQECYAFNGTQRxxFLERYIYNREEYARFDSDVGEFRAVTELGRPAAEYWNSQ

65  KDILERKRAAVDRVCRHNYQLELRTTLQRRVEPTVTISPSRTEALNHHNLLVCSVTDFYPAQ
    KDILE KRA  DRVCRHNY+L+   TLQRRV+P V +SPS    L HHNLLVC VTDFYP
63  KDILEEKRAVPDRVCRHNYELDEAVTLQRRVQPKVNVSPSKKGPLQHHNLLVCHVTDFYPGS

127 IKVRWFRNDQEETAGVVSTPLIRNGDWTFQILVMLEMTPQRGDVYTCHVEHPSLQSPITVEWRA
    I+VRWF N QEETAGVVST LIRNGDWTF ILVMLEMTPQ+GDVY C VEH SL SP+TVEW A
125 IQVRWFLNGQEETAGVVSTNLIRNGDWTFxILVMLEMTPQQGDVYICQVEHTSLDSPVTVEWKA
```

Supplemental Table 3 Results of a BLAST analysis between DRA1*01:01, DQA1*01:01 and DPA1*01:03 sequences

DRA1*01:01 vs DPA1*01:03 p=2e-81 Identities:118/187 (63%) Positives: 139/187 (74%)

```
3    IKEEHVIIQAEFYLNPDQSGEFMFDFDGDEIFHVDMAKKETVWRLEEFGRFASFEAQGAL  60
     IK +HV   A F      +GEFMF+FD DE+F+VD+ KKETVW LEEFG+  SFEAQG L
3    IKADHVSTYAAFVQTHRPTGEFMFEFDEDEMFYVDLDKKETVWHLEEFGQAFSFEAQGGL  60

61   ANIAVDKANLEIMTKRSNYTPITNVPPEVTVLTNSPVELREPNVLICFIDKFTPPVVNVT  120
     ANIA+   NL  + +RSN+T  TN PPEVTV    PVEL +PN LIC IDKF PPV+NVT
61   ANIAILNNNLNTLIQRSNHTQATNDPPEVTVFPKEPVELGQPNTLICHIDKFFPPVLNVT  120

121  WLRNGKPVTTGVSETVFLPREDHLFRKFHYLPFLPSTEDVYDCRVEHWGLDEPLLKHWEFDAPSPLP  187
     WL NG+ VT GV+E++FLPR D+ F KFHYL F+PS ED YDCRVEHWGLD+PLLKHWE   P  +P
121  WLCNGELVTEGVAESLFLPRTDYSFHKFHYLTFVPSAEDFYDCRVEHWGLDQPLLKHWEAQEPIQMP  187
```

DRA1*01:01 vs DQA1*01:01 p=8e-77 Identities:99/168 (59%) Positives 122/168 (72%)

```
19   SGEFMFDFDGDEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSN  78
     SG++  +FDGDE F+VD+ +KET WR  EF +F  F+ QGAL N+AV K NL IM KR N
20   SGQYTHEFDGDEQFYVDLERKETAWRWPEFSKFGGFDPQGALRNMAVAKHNLNIMIKRYN  79

79   YTPITNVPPEVTVLTNSPVELREPNVLICFIDKFTPPVVNVTWLRNGKPVTTGVSETVFL  138
         T  TN  PEVTV + SPV L +PN LIC +D   PPVVN+TWL NG+ VT GVSET FL
80   STAATNEVPEVTVFSKSPVTLGQPNTLICLVDNIFPPVVNITWLSNGQSVTEGVSETSFL  139

139  PREDHLFRKFHYLPFLPSTEDVYDCRVEHWGLDEPLLKHWEFDAPSPL  186
      + DH F K  YL FLPS +++YDC+VEHWGLD+PLLKHWE + P+ +
140  SKSDHSFFKISYLTFLPSADEIYDCKVEHWGLDQPLLKHWEPEIPAQM  187
```

DQA1*01:01 vs DPA1*01:03 p=1e-77 Identities:109/187 (58%) Positives:139/187 (74%)

```
3    IVADHVASCGVNLYQFYGPSGQYTHEFDGDEQFYVDLERKETAWRWPEFSKFGGFDPQGA  60
     I ADHV++      Q + P+G++  EFD DE FYVDL++KET W   EF +   F+ QG
3    IKADHVSTYAA-FVQTHRPTGEFMFEFDEDEMFYVDLDKKETVWHLEEFGQAFSFEAQGG  59

61   LRNMAVAKHNLNIMIKRYNSTAATNEVPEVTVFSKSPVTLGQPNTLICLVDNIFPPVVNI  120
     L N+A+  +NLN +I+R N T ATN+ PEVTVF K PV LGQPNTLIC +D  FPPV+N+
60   LANIAILNNNLNTLIQRSNHTQATNDPPEVTVFPKEPVELGQPNTLICHIDKFFPPVLNV  119

121  TWLSNGQSVTEGVSETSFLSKSDHSFFKISYLTFLPSADEIYDCKVEHWGLDQPLLKHWEPEIPAQM  187
     TWL NG+ VTEGV+E+ FL ++D+SF K  YLTF+PSA++ YDC+VEHWGLDQPLLKHWE + P QM
120  TWLCNGELVTEGVAESLFLPRTDYSFHKFHYLTFVPSAEDFYDCRVEHWGLDQPLLKHWEAQEPIQM  186
```

Supplemental Table 4 Allele sharing between interlocus class II eplets and amino acid residues within a 3.5 Ångstrom radius

	14	24	25	26	27	40	42	
	E	V	R	Residue	V	F	S	Eplet
DRB1*03:01	-	-	-	26Y	-	-	-	rq26Y
DRB1*09:01	-	-	-	26Y	-	-	-	rq26Y
DRB3*01:01	-	-	-	26Y	-	-	-	rq26Y
DQB1*03:01	M	-	-	26Y	-	-	-	rq26Y
DQB1*06:01	M	-	-	26Y	-	-	-	rq26Y

	30	35	36	3738	39	48	50	54	
	Y	E	E	Residues	R	R	V	G	Eplet
DRB1*07:01	L	-	-	37F38V	-	-	-	-	rp37FV
DRB1*14:01	-	-	-	37F38V	-	-	-	-	rp37FV
DRB1*14:54	-	-	-	37F38V	-	-	-	-	rp37FV
DRB3*03:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*02:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*03:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*04:02	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*06:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*08:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*09:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*10:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*14:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*16:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*17:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*18:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*19:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*20:01	-	-	-	37F38V	-	-	-	-	rp37FV
DPB1*23:01	-	-	-	37F38V	-	-	-	-	rp37FV

	30	35	36	3738	39	48	50	54	
	Y	E	E	Residues	R	R	V	G	Eplet
DRB1*10:01	R	-	-	37Y38A	-	-	-	-	rqp37YA
DRB3*02:02	H*	-	-	37Y38A	-	-	-	-	rqp37YA
DRB4*01:01	-	-	-	37Y38A	-	Q*	-	-	rqp37YA
DRB4*01:03	-	-	-	37Y38A	-	Q*	-	-	rqp37YA
DQB1*03:01	-	-	-	37Y38A	-	-	-	-	rqp37YA
DQB1*03:02	-	-	-	37Y38A	-	-	-	-	rqp37YA
DQB1*03:03	-	-	-	37Y38A	-	-	-	-	rqp37YA
DQB1*04:01	-	-	-	37Y38A	-	-	-	-	rqp37YA
DQB1*04:02	-	-	-	37Y38A	-	-	-	-	rqp37YA
DQB1*06:02	-	-	-	37Y38A	-	-	-	-	rqp37YA
DQB1*06:03	H*	-	-	37Y38A	-	-	-	-	rqp37YA
DQB1*06:04	H*	-	-	37Y38A	-	-	-	-	rqp37YA
DQB1*06:05	-	-	-	37Y38A	-	-	-	-	rqp37YA
DQB1*06:09	-	-	-	37Y38A	-	-	-	-	rqp37YA
DPB1*01:01	-	-	-	37Y38A	-	-	-	-	rqp37YA
DPB1*11:01	-	-	-	37Y38A	-	-	-	-	rqp37YA
DPB1*13:01	-	-	-	37Y38A	-	-	-	-	rqp37YA
DPB1*15:01	-	-	-	37Y38A	-	-	-	-	rqp37YA

	30	35	36	3738	39	48	50	54	
	Y	E	E	Residues	R	R	V	G	Eplet
DRB1*04:01	-	-	-	37Y38V	-	-	-	-	rq37YV
DRB1*04:02	-	-	-	37Y38V	-	-	-	-	rq37YV
DRB1*04:03	-	-	-	37Y38V	-	-	-	-	rq37YV
DRB1*04:04	-	-	-	37Y38V	-	-	-	-	rq37YV
DRB1*04:05	-	-	-	37Y38V	-	-	-	-	rq37YV
DRB1*08:01	-	-	-	37Y38V	-	-	-	-	rq37YV
DRB1*08:02	-	-	-	37Y38V	-	-	-	-	rq37YV
DRB1*11:01	-	-	-	37Y38V	-	-	-	-	rq37YV
DRB1*11:02	-	-	-	37Y38V	-	-	-	-	rq37YV
DRB1*11:04	-	-	-	37Y38V	-	-	-	-	rq37YV
DRB1*13:03	-	-	-	37Y38V	-	-	-	-	rq37YV
DQB1*05:01	H*	-	-	37Y38V	-	-	-	-	rq37YV
DQB1*05:02	H*	-	-	37Y38V	-	-	-	-	rq37YV

	64	65	66	6770	68	69	71	72	73	
	Q	K	D	Residues	L	E	K	R	A	Eplet
DQB1*04:01	-	-	-	67I70E	-	-	D	-	-	qp67IE
DQB1*04:02	-	-	-	67I70E	-	-	D	-	-	qp67IE
DPB1*01:01	-	-	-	67I70E	-	-	-	-	-	qp67IE
DPB1*02:01	-	-	-	67I70E	-	-	E*	-	-	qp67IE
DPB1*02:02	-	-	-	67I70E	-	-	E*	-	-	qp67IE
DPB1*04:01	-	-	-	67I70E	-	-	-	-	-	qp67IE
DPB1*04:02	-	-	-	67I70E	-	-	-	-	-	qp67IE
DPB1*05:01	-	-	-	67I70E	-	-	-	-	-	qp67IE
DPB1*08:01	-	-	-	67I70E	-	-	E*	-	-	qp67IE
DPB1*09:01	-	-	-	67I70E	-	-	E*	-	-	qp67IE
DPB1*10:01	-	-	-	67I70E	-	-	E*	-	-	qp67IE
DPB1*13:01	-	-	-	67I70E	-	-	E*	-	-	qp67IE
DPB1*17:01	-	-	-	67I70E	-	-	E*	-	-	qp67IE
DPB1*18:01	-	-	-	67I70E	-	-	-	-	-	qp67IE
DPB1*19:01	-	-	-	67I70E	-	-	E*	-	-	qp67IE
DPB1*23:01	-	-	-	67I70E	-	-	-	-	-	qp67IE

	64	65	66	6771	68	69	70	72	73	
	Q	K	D	Residue	L	E	E	R	A	Eplet
DRB1*13:03	-	-	-	67I71K	-	-	D*	-	-	rqp67IK
DQB1*02:01	-	-	-	67I71K	-	-	R	-	-	rqp67IK
DQB1*02:02	-	-	-	67I71K	-	-	R	-	-	rqp67IK
DPB1*01:01	-	-	-	67I71K	-	-	-	-	-	rqp67IK
DPB1*04:01	-	-	-	67I71K	-	-	-	-	-	rqp67IK
DPB1*04:02	-	-	-	67I71K	-	-	-	-	-	rqp67IK
DPB1*05:01	-	-	-	67I71K	-	-	-	-	-	rqp67IK
DPB1*18:01	-	-	-	67I71K	-	-	-	-	-	rqp67IK

	64	65	66	6771	68	69	70	72	73	
	Q	K	D	Residues	L	E	Q	R	G	Eplet
DRB1*03:01	-	-	-	67L71K	-	-	-	-	-	rq67LK
DRB1*03:02	-	-	-	67L71K	-	-	-	-	-	rq67LK
DRB1*04:01	-	-	-	67L71K	-	-	-	-	A	rq67LK
DRB3*01:01	-	-	-	67L71K	-	-	-	-	-	rq67LK
DRB3*0201	-	-	-	67L71K	-	-	-	-	-	rq67LK
DRB3*02:02	-	-	-	67L71K	-	-	-	-	-	rq67LK
DRB3*03:01	-	-	-	67L71K	-	-	-	-	-	rq67LK
DPB1*03:01	-	-	-	67L71K	-	-	E*	-	A	rq67LK
DPB1*14:01	-	-	-	67L71K	-	-	E*	-	A	rq67LK
DPB1*28:01	-	-	-	67L71K	-	-	E*	-	A	rq67LK

	64	65	66	6771	68	69	70	72	73	
	Q	K	D	Residues	L	E	Q	R	A	Eplet
DRB1*01:01	-	-	-	67L71R	-	-	-	-	-	rp67LR
DRB1*0102	-	-	-	67L71R	-	-	-	-	-	rp67LR
DRB1*04:03	-	-	-	67L71R	-	-	-	-	-	rp67LR
DRB1*04:05	-	-	-	67L71R	-	-	-	-	-	rp67LR
DRB1*10:01	-	-	-	67L71R	-	-	R*	-	-	rp67LR
DRB1*14:01	-	-	-	67L71R	-	-	R*	-	-	rp67LR
DRB1*14:02	-	-	-	67L71R	-	-	-	-	-	rp67LR
DRB4*01:01	-	-	-	67L71R	-	-	R*	-	-	rp67LR
DPB1*11:01	-	-	-	67L71R	-	-	E*	-	-	rp67LR

	70	71	72	73	7475	76	77	78	79	80	
	Q	K	R	A	Residues	D	T	Y	C	R	Eplet
DRB1*01:01/02	-	R*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*01:03	D	E*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*04:01	-	-	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*04:02	D	E*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*04:04/05	-	R*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*10:01	R*	R*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*11:01/04	D	R*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*11:02	D	E*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*12:01/02	D	R*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*13:01/02	D	E*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*13:03	D	-	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*14:02	-	R*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*15:01/02/03	-	A	-	-	74A75V	-	-	-	-	-	rq74AV
DRB1*16:01/02	D	R*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB5*01:01	D	R*	-	-	74A75V	-	-	-	-	-	rq74AV
DRB5*02:02	-	A	-	-	74A75V	-	-	-	-	-	rq74AV
DQB1*02:01	R*	-	-	-	74A75V	-	R	V	-	-	rq74AV
DQB1*02:02	R*	-	-	-	74A75V	-	R	V	-	-	rq74AV

	73	74	75	76	7778	79	80	81	
	A	V	P	D	Residues	C	H	N	Eplet
DQB1*02:01	-	A*	V	-	77R78V	-	-	-	qp77RV
DQB1*02:02	-	A*	V	-	77R78V	-	-	-	qp77RV
DQB1*05:01	-	S	V	-	77R78V	-	-	-	qp77RV
DQB1*05:02	-	S	V	-	77R78V	-	-	-	qp77RV
DPB1*01:01	-	-	-	-	77R78V	-	-	-	qp77RV
DPB1*03:01	-	-	-	-	77R78V	-	-	-	qp77RV
DPB1*08:01	-	-	-	-	77R78V	-	-	-	qp77RV
DPB1*09:01	-	-	-	-	77R78V	-	-	-	qp77RV
DPB1*10:01	-	-	-	-	77R78V	-	-	-	qp77RV
DPB1*14:01	-	-	-	-	77R78V	-	-	-	qp77RV

	73	74	75	76	7778	79	80	81	
	A	E	L	D	Residues	C	H	N	Eplet
DRB1*07:01	G	Q*	V*	-	77T78V	-	-	-	rq77TV
DRB1*09:01	-	-	V*	-	77T78V	-	-	-	rq77TV
DRB1*09:02	-	-	V*	-	77T78V	-	-	-	rq77TV
DQB1*03:01	-	-	-	-	77T78V	-	-	-	rq77TV
DQB1*03:02	-	-	-	-	77T78V	-	-	-	rq77TV
DQB1*03:03	-	-	-	-	77T78V	-	-	-	rq77TV
DQB1*04:01	-	S	V*	-	77T78V	-	-	-	rq77TV
DQB1*04:02	-	S	V*	-	77T78V	-	-	-	rq77TV
DQB1*06:01	-	-	-	-	77T78V	-	-	-	rq77TV
DQB1*06:02	-	-	-	-	77T78V	-	-	-	rq77TV
DQB1*06:03	-	-	-	-	77T78V	-	-	-	rq77TV
DQB1*06:04	-	-	-	-	77T78V	-	-	-	rq77TV
DQB1*06:05	-	-	-	-	77T78V	-	-	-	rq77TV
DQB1*06:09	-	-	-	-	77T78V	-	-	-	rq77TV

	139	141	140142	143	159	160	
	K	G	Residues	V	V	M	Eplet
DRB1*03:01	-	-	140T142V	-	-	-	rq140TV
DRB1*03:02	-	-	140T142V	-	-	-	rq140TV
DRB1*04:01	-	-	140T142V	-	-	-	rq140TV
DRB1*04:02	-	-	140T142V	-	-	-	rq140TV
DRB1*04:03	-	-	140T142V	-	-	-	rq140TV
DRB1*04:04	-	-	140T142V	-	-	-	rq140TV
DRB1*04:05	-	-	140T142V	-	-	-	rq140TV
DRB1*08:01	-	-	140T142V	-	-	-	rq140TV
DRB1*08:02	-	-	140T142V	-	-	-	rq140TV
DRB1*10:01	-	-	140T142V	-	-	-	rq140TV
DRB1*11:01	-	-	140T142V	-	-	-	rq140TV
DRB1*11:02	-	-	140T142V	-	-	-	rq140TV
DRB1*11:04	-	-	140T142V	-	-	-	rq140TV
DRB1*12:01	-	-	140T142V	-	-	-	rq140TV
DRB1*12:02	-	-	140T142V	-	-	-	rq140TV
DRB1*13:01	-	-	140T142V	-	-	-	rq140TV
DRB1*13:02	-	-	140T142V	-	-	-	rq140TV
DRB1*13:03	-	-	140T142V	-	-	-	rq140TV
DRB1*14:01	-	-	140T142V	-	-	-	rq140TV
DRB1*14:02	-	-	140T142V	-	-	-	rq140TV
DRB1*14:54	-	-	140T142V	-	-	-	rq140TV
DRB3*03:01	-	-	140T142V	-	-	-	rq140TV
DQB1*03:01	T	-	140T142V	-	-	-	rq140TV
DQB1*03:02	T	-	140T142V	-	-	-	rq140TV
DQB1*03:03	T	-	140T142V	-	-	-	rq140TV
DQB1*04:01	T	-	140T142V	-	-	-	rq140TV
DQB1*04:02	T	-	140T142V	-	-	-	rq140TV

	27	32	33	34	35	36	47	
	H	D	E	Residue	F	Y	C	Eplet
DQA1*0102	-	-	-	34Q	-	-	R	pq34Q
DQA1*0103	-	-	-	34Q	-	-	R	pq34Q
DQA1*0401	-	-	-	34Q	-	-	-	pq34Q
DQA1*0501	-	-	-	34Q	-	-	-	pq34Q
DQA1*0503	-	-	-	34Q	-	-	-	pq34Q
DQA1*0505	-	-	-	34Q	-	-	-	pq34Q
DQA1*0601	-	-	-	34Q	-	-	-	pq34Q
DPA1*0201	F	-	-	34Q	-	-	H	pq34Q
DPA1*0202	F	-	-	34Q	-	-	H	pq34Q

Supplemental Table 5 Amino acid residues within a 3.5 Ångstroms of β chain eplets that are monomorphic at other loci

	12	27	Eplet	29	39	40	47	
All DPB1	R	L	28E	R	R	F	F	
DRB1*01:01	K*	-	28E	-	-	-	Y*	monoP
DRB1*01:02	K*	-	28E	-	-	-	Y*	monoP
DRB1*01:03	K*	-	28E	-	-	-	Y*	monoP
DRB1*03:02	T	-	28E	-	-	-	Y*	monoP
DRB1*07:01	K*	-	28E	-	-	-	Y*	monoP
DRB1*10:01	K*	-	28E	-	-	Y*	Y*	monoP
DRB1*12:01	T	-	28E	-	-	-	Y*	monoP
DRB1*14:02	T	-	28E	-	-	-	Y*	monoP
DRB3*02:02	K*	-	28E	-	-	-	Y*	monoP
DRB3*03:01	K*	-	28E	-	-	-	Y*	monoP

	103	Eplet	105	106	107	114	
All DQB	P	104S	R	T	E	L	
DRB1*01	-	104S	K*	`	Q*	-	monoQ
DRB1*03	-	104S	K*	`	Q*	-	monoQ
DRB1*08	-	104S	K*	`	Q*	-	monoQ
DRB1*10	-	104S	K*	`	Q*	-	monoQ
DRB1*11	-	104S	K*	`	Q*	-	monoQ
DRB1*12	-	104S	K*	`	Q*	-	monoQ
DRB1*13	-	104S	K*	`	Q*	-	monoQ
DRB1*14	-	104S	K*	`	Q*	-	monoQ
DRB1*15	-	104S	K*	`	Q*	-	monoQ
DRB1*16	-	104S	K*	`	Q*	-	monoQ
DRB4	-	104S	K*	`	Q*	-	monoQ

	132	Eplet	134	135	
All DPB	F	133L	N	G	
DRB1*15:01	-	133L	-	-	monoP
DRB1*16:01	-	133L	-	-	monoP

	9	10	29	Eplet	36	37	38	
All DPB1	Y	Q	R	30YI	E	Y	A	
DRB4*01:01	E	-	-	30YI	-	-	-	monoP
DQB1*0301	-	-	-	30YI	-	-	-	monoP
DQB1*0302	-	-	-	30YI	-	-	-	monoP
DQB1*0303	-	-	-	30YI	-	-	-	monoP
DQB1*0401	F*	-	-	30YI	-	-	-	monoP
DQB1*0402	F*	-	-	30YI	-	-	-	monoP
DQB1*0601	L	-	-	30YI	-	D	-	monoP
DQB1*0602	F*	-	-	30YI	-	-	-	monoP
DQB1*0605	-	-	-	30YI	-	-	-	monoP
DQB1*0609	-	-	-	30YI	-	-	-	monoP

	40	41	44	Eplet	47	48	
All DRB1	F	D	V	45GE	F	R	
All DPB1	F	D	V	45GE	F	R	
DQB1*0201	-	-	-	45GE	-	-	monoRP
DQB1*0202	-	-	-	45GE	-	-	monoRP

	101	102	115	Eplet	117	160		
All DPB	**V***	**S**	**L**	**116V**	**C**	**M**		
nonDQB1*05	**I***	**-**	**-**	**116V**	**-**	**-**	**monoP**	

	138	139	Eplet	141	142	143	144	145	
All DPB1	E	T	140A	G	V	V	S	T	
DQB1*02:01	-	-	140A	-	-	-	-	-	monoP
DQB1*02:02	-	-	140A	-	-	-	-	-	monoP
DQB1*05:01	-	-	140A	-	-	-	-	-	monoP
DQB1*05:02	-	-	140A	-	-	-	-	-	monoP
DQB1*06:01	-	-	140A	-	-	-	-	-	monoP
DQB1*06:02	-	-	140A	-	-	-	-	-	monoP
DQB1*06:03	-	-	140A	-	-	-	-	-	monoP
DQB1*06:04	-	-	140A	-	-	-	-	-	monoP
DQB1*06:05	-	-	140A	-	-	-	-	-	monoP
DQB1*06:09	-	-	140A	-	-	-	-	-	monoP

Supplemental Table 6 Amino acid residues within a 3.5 Ångstroms of alpha chain eplets that are monomorphic at other loci

	36	43	44	Eplet	46	
All DPA1	Y	E	T	45V	W	
non DQA1*01	-	-	-	45V	-	monoP

	57	58	59	60	Eplet	62	63	64	
DRA1* 01:01	F	E	A	Q	61G	A	L	A	
DQA1*01	-	D*	P	-	61G	-	-	-	monoR

	61	62	63	64	65	Eplet	67	68	69	
DRA1* 01:01	G	A	L	A	N	66I	A	V	D	
DQA1*0201	F	-	-	T	-	66I	-	-	L	monoR
DQA1*0301	F	-	-	T	-	66I	-	-	L	monoR
DQA1*0302	F	-	-	T	-	66I	-	-	L	monoR
DQA1*0303	F	-	-	T	-	66I	-	-	L	monoR
DQA1*0401	F	-	-	T	-	66I	-	-	T*	monoR
DQA1*0402	F	-	-	T	-	66I	-	-	T*	monoR
DQA1*0404	F	-	-	T	-	66I	-	-	T*	monoR
DQA1*0501	F	-	-	T	-	66I	-	-	L	monoR
DQA1*0502	F	-	-	T	-	66I	-	-	L	monoR
DQA1*0503	F	-	-	T	-	66I	-	-	L	monoR
DQA1*0504	F	-	-	T	-	66I	-	-	L	monoR
DQA1*0505	F	-	-	T	-	66I	-	-	L	monoR
DQA1*0601	F	-	-	T	-	66I	-	-	T*	monoR
DQA1*0602	F	-	-	T	-	66I	-	-	T*	monoR

	76	77	78	79	Eplet	81	82	
DRA1* 01:01	M	T	K	R	80S	N	Y	
DQA1*0201	L*	I	-	-	80S	-	S	monoR
DQA1*0301	V*	I	-	-	80S	-	S	monoR
DQA1*0302	V*	I	-	-	80S	-	S	monoR
DQA1*0303	V*	I	-	-	80S	-	S	monoR
DQA1*0401	L*	I	-	-	80S	-	S	monoR
DQA1*0402	L*	I	-	-	80S	-	S	monoR
DQA1*0404	L*	I	-	-	80S	-	S	monoR
DQA1*0501	L*	I	-	-	80S	-	S	monoR
DQA1*0502	L*	I	-	-	80S	-	S	monoR
DQA1*0503	L*	I	-	-	80S	-	S	monoR
DQA1*0504	L*	I	-	-	80S	-	S	monoR
DQA1*0505	L*	I	-	-	80S	-	S	monoR
DQA1*0601	L*	I	-	-	80S	-	S	monoR
DQA1*0602	L*	I	-	-	80S	-	S	monoR

	106	155	Eplet	157	
DRA1* 01:01	N	P	156F	L	
nonDQA1*05	-	T*	156F	-	monoR

	127	159	Eplet	162	
Al DPA1	**N**	**S**	**160AE**	**E**	
DQA1*0501	-	-	160AE	D*	monoP
DQA1*0505	-	-	160AE	-	monoP

	173	174	Eplet	176	
DRA1* 01:01	**L**	**D**	**175E**	**P**	
DQA1*02:01	-	-	175E	-	monoR
DQA1*03:01	-	-	175E	-	monoR
DQA1*03:02	-	-	175E	-	monoR
DQA1*03:03	-	-	175E	-	monoR
DQA1*04:01	-	-	175E	-	monoR
DQA1*04:02	-	-	175E	-	monoR
DQA1*04:04	-	-	175E	-	monoR
DQA1*06:01	-	-	175E	-	monoR
DQA1*06:02	-	-	175E	-	monoR

	173	174	Eplet	176	
All DPA1	**L**	**D**	**175Q**	**P**	
DQA1*01:01	-	-	175Q	-	monoP
DQA1*01:02	-	-	175Q	-	monoP
DQA1*01:03	-	-	175Q	-	monoP
DQA1*01:04	-	-	175Q	-	monoP
DQA1*01:05	-	-	175Q	-	monoP
DQA1*01:06	-	-	175Q	-	monoP
DQA1*01:07	-	-	175Q	-	monoP

	85	Eplet	87	88	116	
All DQA1	**A**	**86T**	**N**	**D**	**F**	
DPA1*01:03	-	86T	-	-	-	monoQ
DPA1*01:04	-	86T	-	-	-	monoQ
DPA1*03:01	-	86T	-	-	-	monoQ

Supplemental Table 7 Residue analysis of class II eplets with subscript notations

DQB Eplet	New Name	Alleles	Unique Polymorphic Residues										(ElliPro scores)								Monomorphic Residued				Interlocus Residues	
45GE$_3$	52LL	DQ2	52L	0.889	55L	0.889	28S	0.258	30S	0.272	37I	0.449									46E	47F			71K	74A
46VY$_2$	46VY	non-DQ2	46V	0.814	52P	0.889	28T	0.258													47Y					
52PQ$_2$	52PQ	DQ5,6	53Q	0.738	89G	0.456	90I	0.243													84E	85V				
56L$_2$	56L	DQ4	56L	0.814	71D	0.529															70E					
71A$_2$	116L	DQ5	116I	0.394	125S	0.120	14L	0.111																	71A	
84QL$_3$	84QL	non-DQ56	84Q	0.742	86E	0.338	87L	0.445													53L	85L				
			89T	0.456	90T	0.243	125A	0.120																		
140A$_2$	182S	DQ2,5,6	182S	0.863																	140A					
140T$_2$	182N	DQ3,4	182N	0.863																					140T	
DQA Eplet																										
40GR$_3$	40GR	DQA456	40G	0.700	47C	0.166	50V	0.419	51L	0.292															53Q	
40E$_2$	40E	DQA123	40E	0.700																	51F					
52SK$_5$	52SK	DQA1	52S	0.580	53K	0.740	18F	0.599	45A	0.321	48W	0.201									47R	50E				
			11C	0.147	55G	0.602	56G	0.643	64R	0.499											61G	175Q				
			66M	0.296	69A	0.293	76M	0.563	80Y	0.765																
61FT$_4$	61FT	nonDQA1	18S	0.599	55R	0.602	61F	0.421	64T	0.499											11Y	45V	48L	66I		
75I$_2$	75I	nonDQA5	75I	0.681	156F	0.475	161D	0.960	163I	0.803											80S					
75S$_3$	75S	DQA5	75S	0.681	156L	0.475	163S	0.803	175K	0.909											161E					
76V$_3$	76V	DQA3	76V	0.563	26S	0.055	47Q	0.166	187T	ND																
DRB eplet																										
105R$_2$	108T	DR51	108T	0.946																	14E	96E	105R	191Q		
142M$_3$	142M	DR15,16	142M	0.369																	96Q	133L				
25Q$_3$	25Q	DR7	25Q	0.173	30L	0.349	14K	0.030																	11G	
37D$_2$	30D	DRB5*01:01	30D	0.349																	31I				37D	
48Q$_6$	48Q	DR53	48Q	0.661	18L	0.285	25W	0.173	26N	0.149																
			41N	0.324	44L	0.624	81Y	0.754	187Q	ND																
6C$_2$	6C	DRB5*02:02	6C	0.978																	157I					
96Y$_2$	96Y	DR4	96Y	0.447	33H	0.908															180L					