HLA Interlocus Class II Eplet Database

The 2019 update of the HLA Epitope Registry includes a new database for antibody-verified and predicted interlocus class II eplets. While many studies have demonstrated that HLA-DR, -DQ and -DP matching at the eplet level reduces allograft rejection and improves transplant outcome, these studies have generally examined the eplet effect for individual class II loci. Until now, little attention has been given to so-called interlocus eplets shared between HLA-DR, -DQ and/or -DP alleles.

The registry already describes several antibody-verified DRB eplets 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). Similarly, there are well-described antibody-verified class I eplets shared by combinations of HLA-A, -B and/or -C alleles such as 62GE (on A2, B57 and B58), 82LR (on A23, A24, A25, A32 and Bw4-carrying HLA-B alleles) and 163LW (shared between various HLA-A, -B and -C alleles). Except for the well-documented, antibody-verified eplet shared between DR11 and a group of DPB alleles, there is little information about interlocus eplet sharing between class II loci.

Comparisons of DRB, DQ and DP amino acid sequences were performed using the protein BLAST (Basic Local Alignment Search Tool) program, a commonly used tool to determine sequence and structural similarities between different proteins. This program can be accessed at https://blast.ncbi.nlm.nih.gov and upon entering amino acid sequences of two proteins it generates the following information: (1) Statistical significance of the residue alignment between the 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. 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, 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.

A BLAST analysis of HLA-DR, HLA-DQ and HLA-DP proteins shows, as expected, a very high degree of sequence homology. However, the HLA-DPB sequences contain gaps in sequence positions 24 and 25 when compared with HLA-DRB and HLA-DQB, and

there is also a gap in position 154. Interlocus eplets were determined for the position 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 on DPB, adjustments were made whereby DPB positions 24-153 became 26-155 and DPB positions 154-190 because 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. Eplet names were assigned with prefixes such as “rq”, “rp”, “qp” or “rqp” to indicate the sharing between DRB, DQB and/or DPB alleles.

A similar analysis was also performed to identify interlocus eplets on class II α chains. This was done for DRA, DQA and DPA based on the 1-190 sequence of DQA alleles; 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; those eplet names carry the “qp” prefix.

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, we defined eplet structures by identifying with the Cn3D program all residues within a 3.5 Ångstrom radius of the polymorphic residue(s).

The new database describes the experimental evidence for five antibody-verified interlocus class II eplets shared by DRB, DQB and/or DPB; their sequence positions within a 3.5 Ångstrom radius show high degrees of identical residue sharing. A search of the entire 1-190 amino acid residue sequence has identified an additional sixteen interlocus class II eplets shared by DRB, DQB and/or DPB with comparably similar degrees of residue sharing between eplet-carrying alleles. Certain interlocus class II 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 (e.g., 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. At this time, no interlocus eplets shared by DQA and DPA have been described in the epitope registry.

We believe the inclusion of interlocus class II eplet comparisons will enhance the efficiency of eplet-based class II matching. 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.