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DOI:
10.1097/TP.0000000000000546

Document Version
Peer reviewed version

Citation for published version (Harvard):
Mallon, DH, Bradley, JA, Winn, PJ, Taylor, CJ & Kosmoliaptsis, V 2015, 'Three-dimensional structural modelling and calculation of electrostatic potentials of HLA Bw4 and Bw6 epitopes to explain the molecular basis for alloantibody binding: toward predicting HLA antigenicity and immunogenicity', Transplantation, vol. 99, no. 2, pp. 385-90. https://doi.org/10.1097/TP.0000000000000546

Three-dimensional structural modelling and calculation of electrostatic potentials of HLA Bw4 and Bw6 epitopes to explain the molecular basis for alloantibody binding: towards predicting HLA antigenicity and immunogenicity

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Funding

The study was funded in part by the NIHR Cambridge Biomedical Research Centre.

Authors' Contribution

DH Mallon: Participated in research design, performance of research, data analysis and writing of the manuscript

JA Bradley: Participated in research design, data analysis and writing of the manuscript

PJ Winn: Participated in research design and reviewed the manuscript

CJ Taylor: Participated in research design, data analysis and writing of the manuscript

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Conflicts of interest

The authors declare no conflicts of interest.

Abbreviations

ESD: Electrostatic Similarity Distance

HLA: Human Leukocyte Antigen

mAb: monoclonal antibody

3-D: Three-dimensional

Abstract

Background: We have previously shown that qualitative assessment of surface electrostatic potential of HLA class I molecules helps explain serological patterns of alloantibody binding. We have now used a novel computational approach to quantitate differences in surface electrostatic potential of HLA B-cell epitopes, and applied this to explain HLA Bw4 and Bw6 antigenicity.

Methods: Protein structure models of HLA class I alleles expressing either the Bw4 or Bw6 epitope (defined by sequence motifs at positions 77-83) were generated using comparative structure prediction. The electrostatic potential in three-dimensional space encompassing the Bw4/Bw6 epitope was computed by solving the Poisson-Boltzmann equation and quantitatively compared in a pairwise, all-versus-all, fashion to produce distance matrices that cluster epitopes with similar electrostatics properties.

Results: Quantitative comparison of surface electrostatic potential at the carboxyl terminal of the α1-helix of HLA class I alleles, corresponding to amino acid sequence motif 77-83, produced clustering of HLA molecules in three principal groups according to Bw4 or Bw6 epitope expression. Remarkably, quantitative differences in electrostatic potential reflected known patterns of serological reactivity better than Bw4/Bw6 amino acid sequence motifs. Quantitative assessment of epitope electrostatic potential allowed the impact of known amino acid substitutions (HLA-B*07:02 R79G, R82L, G83R) that are critical for antibody binding to be predicted.

Conclusion: We describe a novel approach for quantitating differences in HLA B-cell epitope electrostatic potential. Proof of principle is provided that this approach enables better assessment of HLA epitope antigenicity than amino acid sequence data alone and it may allow prediction of HLA immunogenicity.

Introduction

HLA mismatched allografts commonly provoke alloantibody responses directed against polymorphic amino acid motifs (B-cell epitopes) on the surface of donor HLA glycoproteins. Such B-cell responses are often refractory to conventional immunosuppressive agents and are a major cause of chronic graft rejection. The alloantibody response in recipients of grafts with multiple HLA mismatches is usually directed against a small number (typically one or two) of immunodominant epitopes (1, 2). However, because polymorphic HLA molecules have evolved from common ancestral HLA types many epitopes are shared by different HLA specificities resulting in a high degree of serological cross-reactivity. For a potential transplant recipient with a given HLA type, the ability to predict the relative immunogenicity to different HLA alloantigens would enable a more rational approach to donor selection, thereby avoiding HLA mismatches most likely to evoke a strong alloantibody response.

The immunogenicity of HLA class I and class II mismatches can be predicted by interlocus subtraction of amino acid sequence motifs (triplets and eplets) that define immunogenic epitopes associated with alloantibody production and renal transplant outcome (3-5). However, defining epitopes based on amino acid sequence comparison alone provides an incomplete description of the immunogenicity of an epitope. The specificity and affinity of antibody-antigen interactions are largely governed by electrostatic forces dictated by the number and distribution of charged atoms on the surface of the HLA molecule (6-8). Moreover, amino acid polymorphisms outwith an epitope may alter its tertiary structure and electrostatic pattern (9). Consequently epitopes with an identical amino acid sequence expressed on different HLA molecules may present widely differing electrostatic patterns because of topographical alterations imposed by distant amino acid polymorphisms (9). Conversely, common structural electrostatic motifs may be conserved between HLA molecules despite variation in the amino acid sequence motif because of conservative amino acid substitutions with side chains that have similar physiochemical properties.

We have previously used atomic resolution structural modelling to define the surface electrostatic potential of HLA class I molecules in order to understand better the molecular basis for alloantibody binding epitopes and to predict their relative ability to evoke a humoral response (9). This approach provided novel qualitative insights into the heterogeneity of HLA-specific antibody binding that could not be explained by amino acid sequence comparisons alone. We have now applied methods to quantitatively assess differences in the surface electrostatic patterns of HLA molecules and here we report our analysis of the two common HLA class I epitopes Bw4 and Bw6 that are serologically well-characterised with known amino acid sequence motifs.

Materials and Methods

Generation of structural and physiochemical models of HLA class I molecules.
HLA class I structures, resolved using X-ray crystallography with a resolution of less than 1.5 Å (PDB codes: 1K5N, 1X7Q, 1XH3, 2BVP, 3BWA, 3LN4, 3MRE, 3SPV), were used as templates to generate atomic resolution 3-D structural models of common HLA-A and –B alleles that express the Bw4 or Bw6 epitope, as previously described (9). HLA allele sequence data was retrieved from the IMGT/HLA database (ftp.ebi.ac.uk/pub/databases/ipd/imgt/hla). Mean sequence homology between templates and target sequences was 91.9% (range: 84.1%-100.0%). Because electrostatic forces are a critical determinant of antibody-antigen interaction, the electrostatic potential above the

molecular surface of each HLA allele modelled was calculated (10). To standardise the peptide binding groove environment, all HLA class I structures were modelled with an alanine nonamer peptide. In brief, homology modelling was performed using the MODELLER computer algorithm (https://salilab.org/modeller/) (11) and the stereochemical quality of each model confirmed using Ramachandran plot (12), DOPE (13), Verify3D (14) and WHAT_CHECK (15) scores. Atom charges and radii were assigned and side-chains protonated for pH 7.4 using the PARSE force-field in PDB2PQR (16). The electrostatic potential in 3-D space of each HLA class I model was calculated by solving the linearised Poisson-Boltzmann equation in APBS (http://www.poissonboltzmann.org/) (10) for a cubic grid with sides of 353 points at a spacing of 0.33 Å (Figure 1). Other parameters were set as follows: ionic solution of 0.15 M of univalent positive and negative ions; protein dielectric of 2; solvent dielectric of 78; temperature of 310 K; and a probe radius of 1.4 Å.

Quantitative comparison of 3-D electrostatic potential of the Bw4 and Bw6 epitopes
Electrostatic potential comparisons were performed based on the method described by Wade et al (http://pipsa.eml.org/pipsa/) (17, 18). In brief, this method considers the electrostatic potential in a 'skin' above the molecular surface of a protein and quantitative comparison is performed for grid points within the intersection of the 'skins' of two superimposed proteins (18). For the purpose of this study, a 'skin' of 4 Å thickness and raised 3 Å above the molecular surface of HLA molecules was defined. To enable selective comparison of Bw4 and Bw6 epitopes, a spherical region of interest was considered that encompasses the canonical Bw4/Bw6 sequence motif. The centre of the sphere was defined as the geometric average of the position of the side chain atoms of amino acids 77-83 and a radius of 10 Å was selected to encompass the Bw4/Bw6 residues of all superimposed HLA molecules. Electrostatic potential comparisons were made between grid points within the intersecting skins that were bounded by the sphere (Figure 1). This resulted in a median of 2640 grid point comparisons which were then used to calculate a similarity index (using the Hodgkin's index (18, 19)) for the two epitopes being compared. The Hodgkin's index assigns values between 1 (electrostatic identity, both in magnitude and sign) and -1 (electrostatic anticorrelation of the sign of the potential but of the same magnitude), which were then convertedinto a distance (Electrostatic Similarity Distance [ESD] [(2-2SI)½]) to give values between 0 (electrostatic identity) and 2 (electrostatic anti-correlation) where 1 represents no apparent correlation. ESD was considered to 3 decimal places. For the purpose of this study, the electrostatic potential space overlaying the Bw4/Bw6 epitope was sampled using a sphere to identify relevant grid points; different approaches for sampling the electrostatic potential space (e.g. sphere radius of 8-12 Å, utilising a cone instead of a sphere and sampling from a skin of variable thickness and distance from the molecular surface) did not alter the results of the quantitative epitope comparisons (data not shown).

Electrostatic potential comparisons were made for all possible combinations of common Bw4/Bw6 expressing HLA class I alleles studied (in a pairwise, all-versus-all, fashion). The ESDs generated by the epitope comparisons were compiled as a distance matrix that was then displayed as a symmetrical heatmap with re-ordering such that alleles with electrostatically similar epitopes cluster together. Symmetrical heatmaps and allele re-ordering were performed in R using complete-linkage hierarchical clustering as implemented in the *hclust* function (20).

Results

A novel approach for comparing electrostatic potential was employed to assess Bw4 and Bw6 epitope antigenicity. Quantitative assessment of the surface electrostatic potential of Bw4 and Bw6 epitopes was undertaken by placing a sphere of 10 Å radius over the epitope to identify the relevant grid points. Paired comparisons of epitope surface electrostatic potential were made for all possible combinations of the 50 common Bw4/Bw6 expressing HLA class I alleles and the results, clustered according to ESD and depicted as a heatmap and dendrogram, are shown in Figure 2. When all alleles were compared there was substantial heterogeneity in ESD ranging from 0.000 to 1.918 (from a possible range of 0.000 to 2.000) with three principal clusters, two of which contained exclusively Bw6 expressing alleles (cluster A and B) and a third cluster (cluster C) of Bw4 expressing alleles. Within the three clusters there was further variation in ESD (cluster A 0.000 to 1.441; cluster B 0.063 to 0.195; cluster C 0.000 to 0.989). Importantly, Bw4 expressing HLA-A alleles located appropriately within cluster C. The ability of ESD to segregate alleles expressing the serologically distinct Bw4 and Bw6 epitopes supports the concept that quantifying the 3-D surface electrostatic potential of an epitope accurately reflects well-characterised antibody binding patterns. The three Bw6 expressing alleles that comprise cluster B, (HLA-B*18:06, -B*46:01 and -B*73:01) are known not to bind Bw6 alloantibodies and it is notable that their epitope has distinct electrostatic properties to all the other Bw6 expressing alleles. We next extended the analysis to include HLA-B*07:02 alleles in which targeted point mutations have been introduced into the Bw6 epitope that have differing effects on Bw6- specific mAb binding (21) (Figure 3). The substitution of asparagine for threonine at position 80 (B*07:02 N80T) does not affect Bw6 mAb binding (21). HLA-B*07:02 N80 and - B*07:02 T80 are both located in cluster A, indicating a limited effect of the mutation on Bw6 surface electrostatic potential. In contrast, substitution of arginine for glycine at position 79 (HLA-B*07:02 R79G), arginine for leucine at position 82 (HLA-B*07:02 R82L) and glycine for arginine at position 83 (HLA-B*07:02 G83R) resulted in abrogation of Bw6 mAb binding (21). These mutated HLA-B*07:02 molecules were displaced from the native -B*07:02, present in cluster A, into cluster B reflecting marked alterations on epitope surface electrostatic potential.

Discussion

There is increasing awareness of the importance of epitope based HLA matching to offset the risk of humoral alloimmunity following renal transplantation. The major focus to date has been on amino acid sequence comparisons of donor and recipient HLA class I and II epitopes and this approach provides a better assessment of immunological compatibility than conventional HLA matching (3, 4, 22). We have shown previously that incorporating a physiochemical analysis of HLA alloantigens further improves predictions of immunogenicity (23-25) and that qualitative structural assessment of the electrostatic topography of HLA B-cell epitopes provides an explanation for serological patterns of HLA specific antibody binding (9). In the present study we have created atomic resolution molecular models of HLA class I and calculated the electrostatic potential on the 3-D surface of two common B-cell epitopes, Bw4 and Bw6, and applied these to understand better the molecular basis for alloantibody binding. Importantly, the novel quantitative approach described here can be used to compare 3-D surface electrostatic differences between HLA Bcell epitopes that may reflect their relative immunogenicity.

We chose to focus our proof of principle analysis on Bw4 and Bw6 because they are widely expressed on different HLA class I molecules and are serologically well-characterised with known amino acid sequence motifs. When we compared the 3-D surface electrostatic potential of the carboxyl terminal of the α1 helix of HLA-A and -B alleles (corresponding to the position of Bw4 and Bw6), we observed that alleles segregated according to their expression of either Bw4 or Bw6 epitopes indicating that B-cell epitopes with common functional characteristics share similar 3-D surface electrostatic properties. In addition, analysis of HLA-B alleles in which targeted point mutations have been introduced into the Bw6 epitope showed that quantitative assessment of B-cell epitope electrostatic potential accurately reflects the functional impact of critical and non-critical amino acid substitutions on antibody binding. These observations validate the concept that quantitative assessment of electrostatic potential of a B-cell epitope has functional relevance and provides insight into alloantibody-HLA interactions that are not explicable in terms of amino acid sequence data alone.

Our quantitative analysis revealed that three alleles expressing the Bw6 amino acid sequence motif had electrostatic properties distinct from those found on the majority of Bw6 expressing alleles. These alleles are known not to bind Bw6 alloantibodies and it is interesting therefore that the epitope they express has distinct electrostatic characteristics. Moreover, within the two major Bw4 and Bw6 clusters there was further electrostatic heterogeneity. Electrostatic forces are key mediators of the affinity of antigen-antibody interactions (6, 8) and it is possible that such variation in electrostatic potential of the same B-cell epitope, when expressed on different HLA molecules, may be an important determinant for the functional outcome of antibody binding. This has obvious implications for understanding the clinical significance of HLA-specific antibodies in transplantation and it will be important to investigate this by subjecting computational predictions of specific HLA-alloantibody interactions to *in vitro* experimental validation.

The interaction of antibody with antigen is a highly complex and dynamic process and it is important to emphasise that modelling this interaction was not the focus of the present study. Instead, our aim was to provide a computational method for comparing the physiochemical characteristics of an epitope as expressed by different HLA class I alleles to predict antigenicity and potentially immunogenicity. We focused our attention on the Bw4/Bw6 epitope of HLA class I but acknowledge that the antibody binding "footprint" on the HLA molecule extends well beyond the "functional" epitope and that antibody/antigen interactions outwith an epitope may be important, particularly for stabilising the antibody/antigen interaction (3, 26, 27). It is also important to note that in this study, an alanine nonamer was modelled in the binding groove. Alanine was selected because it does not impose electrostatic or steric effects enabling, therefore, accurate prediction of HLA structure and epitope comparison between different HLA molecules. In contrast to the critical importance of peptide for recognition by alloreactive T cells, there is limited evidence for the role of peptide on HLA specific alloantibody binding (28). However, we acknowledge that certain peptides presented by the HLA class I molecule may have an effect on Bw4/Bw6 surface electrostatic potential, but this effect is likely to be limited; modelling this interaction would be complex and beyond the scope of this study. Finally, although protein electrostatic properties are the major determinant of antibody binding affinity, other physical properties of biomolecules, such as hydrophobicity, may also influence antibody binding and have only indirectly been taken into account in our analysis (29).

In conclusion, the present study provides proof of concept that atomic resolution modelling and comparison of B-cell epitope 3-D surface electrostatic potential provides a physiochemical explanation for serological patterns of antibody binding. The quantitative comparison of epitope electrostatic potential between different HLA alleles may provide a novel tool for predicting HLA antigenicity and immunogenicity.

Acknowledgements

This study was funded in part by the NIHR Cambridge Biomedical Research Centre.

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Figure 1.

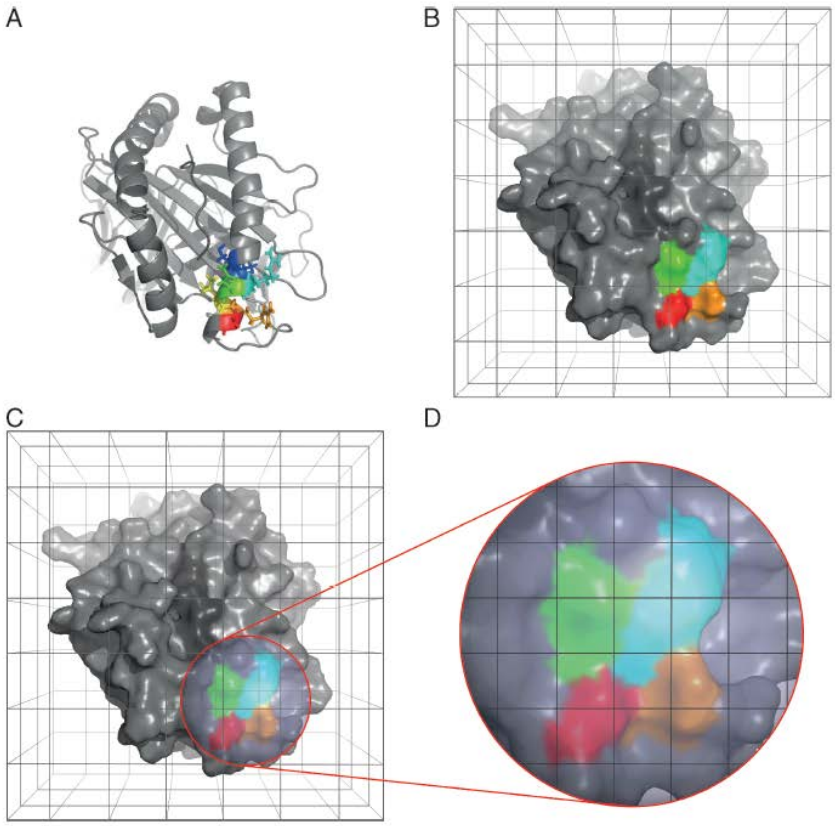

Figure 1. Schematic representation of the method used to quantitate the surface electrostatic potential of the Bw4 and Bw6 epitopes expressed on HLA class I molecules
The HLA class I molecule is depicted in grey and the Bw4 or Bw6 epitope is highlighted in colour. (A) Atomic resolution 3-D structural models of common HLA-A and -B alleles that express the Bw4 or Bw6 epitope were created. (B) The electrostatic potential in the 3-dimensional space around each HLA class I model was calculated by solving the linearised Poisson-Boltzmann equation, as implemented in APBS, for a cubic grid with sides of 353 points spaced 0.33 Å apart. (C and D) To enable selective electrostatic potential comparison of the Bw4 and Bw6 epitopes, a virtual sphere of interest (10 Å in radius) was created to encompass the canonical Bw4/Bw6 motif. Quantitative comparisons of the electrostatic potential of the Bw4/Bw6 epitope were made for each HLA allele by comparing electrostatic potential at analogous grid points within the sphere of interest.

Figure 2.

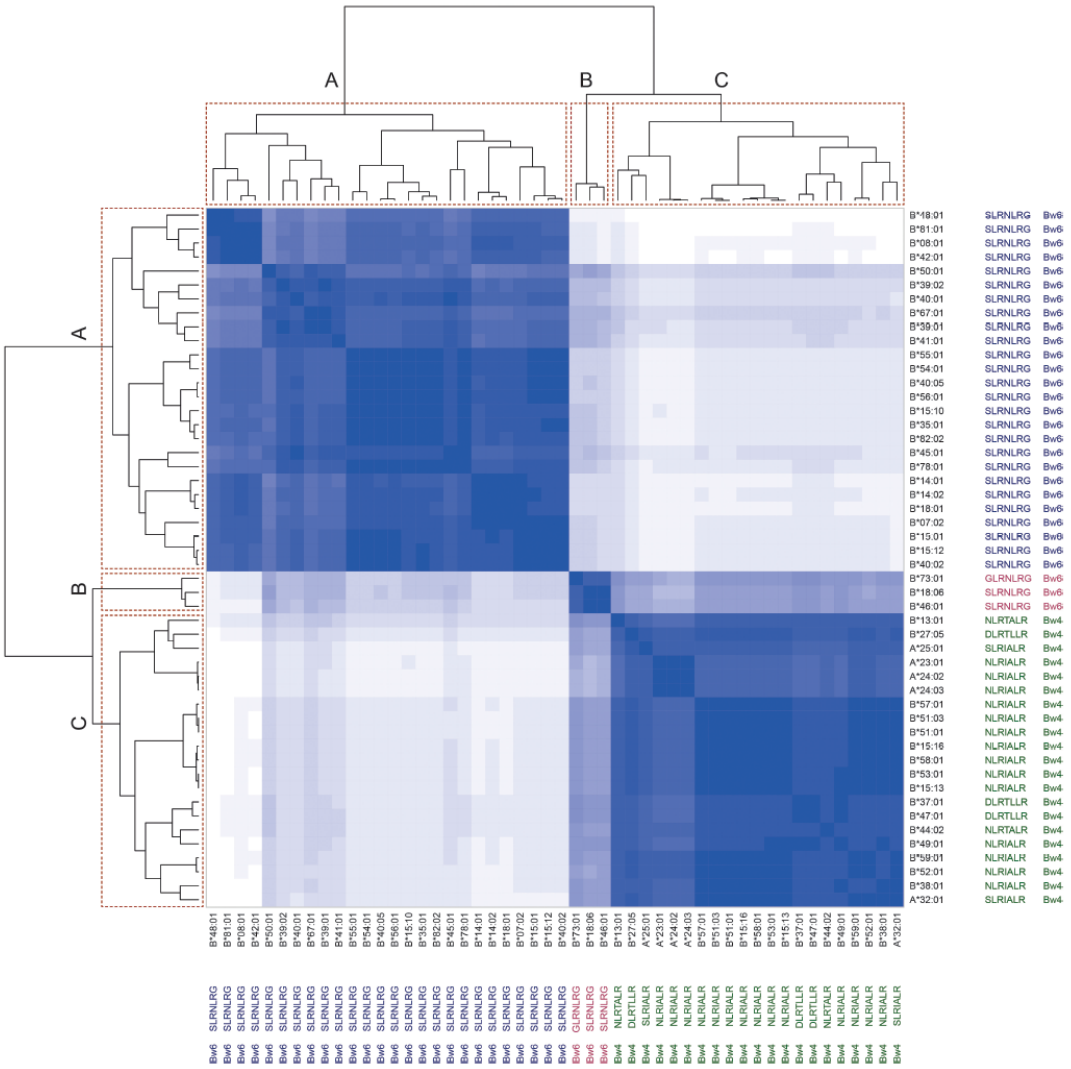

Figure 2. Heatmap and dendrogram of Bw4/Bw6 epitope electrostatic potential similarity between HLA class I alleles

Epitope electrostatic potential comparisons were made for all possible combinations of common Bw4/Bw6 expressing HLA class I alleles (in a pairwise, all-versus-all, fashion). The Electrostatic Similarity Distances (ESD) generated by the epitope comparisons were compiled to form a distance matrix displayed as a symmetrical heatmap with re-ordering such that alleles with electrostatically similar epitopes are clustered together. There was substantial heterogeneity in ESD ranging from 0.000 to 1.918 resulting in three principal clusters, two of which contained exclusively Bw6 expressing alleles (cluster A and B) and a third cluster (cluster C) of Bw4 expressing alleles. White squares represent electrostatic dissimilarity between two epitopes and darker shades of blue represent increasing similarity. The height of the dendrogram arms between a given allele pair is proportional to the electrostatic disparity of their epitopes.

Figure 3.

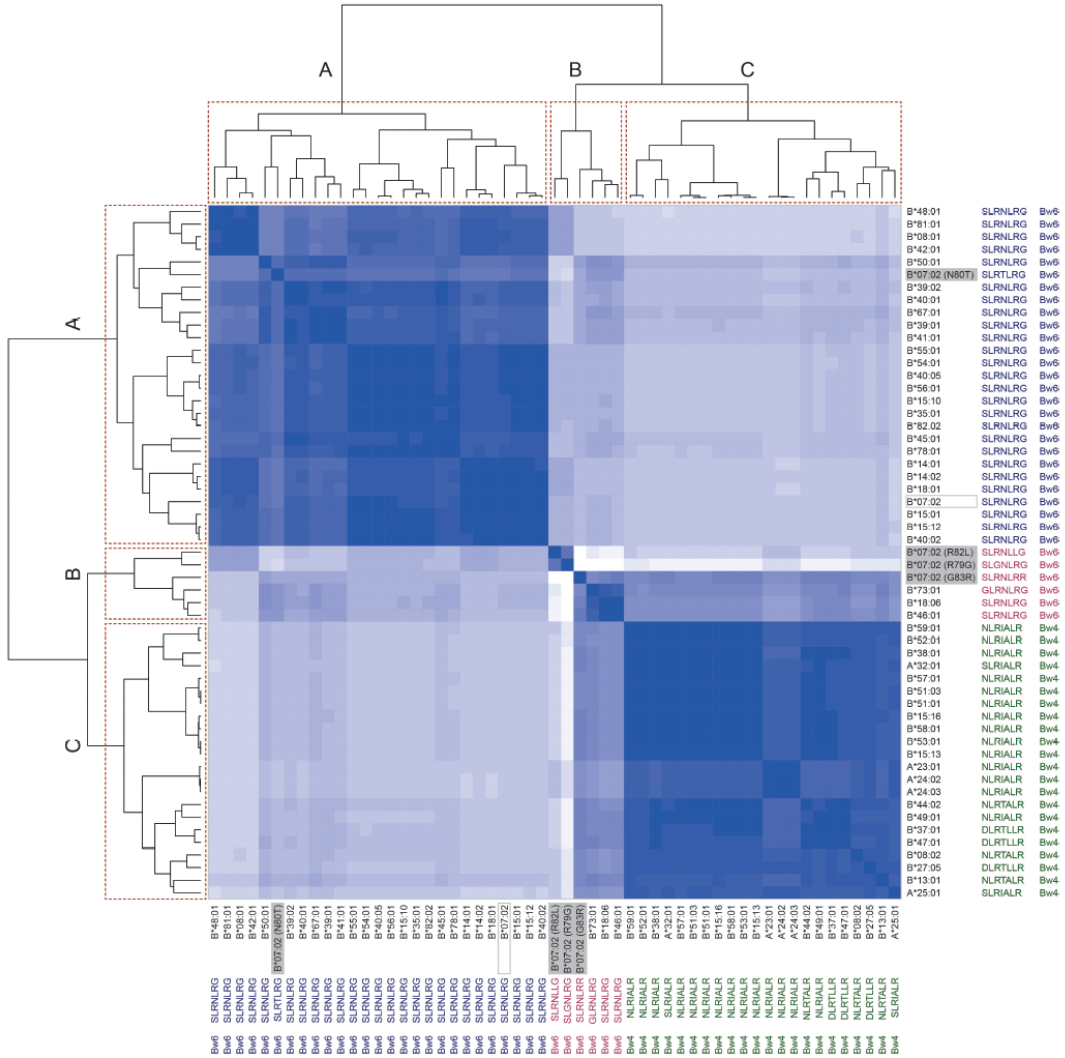

Figure 3. Heatmap and dendrogram of Bw4/Bw6 epitope electrostatic potential similarity between HLA class I alleles including mutant HLA-B*07:02 molecules
Epitope electrostatic potential comparisons were performed for all possible combinations of Bw4/Bw6 expressing HLA class I alleles, including mutant HLA-B*07:02 molecules, in a pairwise, all-versus-all, fashion. The symmetrical heatmap and dendrogram were created as described in Methods and the Figure 2 legend. The amino acid substitution at position 80 (B*07:02 N80T) did not affect Bw6 mAb binding and had a minimal effect on Bw6 epitope electrostatic potential. Amino acid substitutions leading to abrogation of Bw6 mAb binding (HLA-B*07:02 R79G, -B*07:02 R82L and -B*07:02 G83R), highlighted in grey, resulted in marked alterations of epitope surface electrostatic potential.