Structural and electrostatic analysis of HLA B-cell epitopes: inference on immunogenicity and prediction of humoral alloresponses

Dermot H. Mallon[a], *J.A. Bradley*[a], *Craig J. Taylor*[b], *and Vasilis Kosmoliaptsis*[a]

Purpose of review
The immunogenic capacity of donor human leukocyte antigen (HLA) to induce humoral immune responses is not an intrinsic property of the mismatched alloantigen but depends on the HLA phenotype of the recipient. In recent years, advances in molecular sequence technology and information from X-ray crystallography have enabled structural comparison of donor and recipient HLA type providing an opportunity for a more rational approach for determining HLA compatibility. In this article, we review studies investigating the molecular basis of antibody–antigen interactions and present computational approaches to determine the complex physiochemical and structural properties of B-cell epitopes.

Recent findings
The relative immunogenicity of individual HLA mismatches may be predicted from analysis of polymorphic amino acids at continuous and discontinuous HLA sequence positions. The use of alloantigen sequence information alone, however, provides limited insight into key determinants of B-cell epitope immunogenicity, such as the orientation, accessibility and physiochemical properties of amino acid side chains. Advances in computational molecular modelling techniques now enable assessment of HLA-alloantibody interactions at the atomic level. Recent evidence supports a strong link between HLA B-cell epitope surface electrostatic potential and their immunogenicity.

Summary
Assessment of the surface electrostatic properties of HLA alloantigens and computational analyses of HLA-alloantibody interactions represent a promising area for future research into the molecular basis of HLA immunogenicity and antigenicity.

Keywords
antigenicity, computational structure prediction, electrostatics, human leukocyte antigen, immunogenicity

INTRODUCTION

The immune response against an organ allograft is directed principally against mismatched human leukocyte antigen (HLA) glycoproteins expressed on donor tissues. Confrontation of the recipient immune system with donor HLA alloantigens may induce powerful immune responses that lead to acute and/or chronic allograft rejection. Importantly, increasing evidence in recent years supports a central role for humoral alloimmunity in the development of chronic allograft damage [1–4]. In kidney transplantation, minimizing the number of HLA mismatches between the donor and the recipient has a beneficial effect on allograft survival and is the principal reason for the incorporation of HLA matching in national and international organ allocation algorithms [5,6]. However, as a consequence of the extensive polymorphism of the HLA system within and between different ethnic groups, the majority of transplant recipients receive allografts that are mismatched at one or more of the three most widely considered histocompatibility loci, HLA-A, -B and -DR. It has long been recognized that donor HLA alloantigens are not equal in their capacity to induce deleterious humoral immune

[a]Department of Surgery, University of Cambridge, and NIHR Cambridge Biomedical Research Centre and [b]Tissue Typing Laboratory, Cambridge University Hospitals NHS Foundation Trust, Addenbrooke's Hospital, Cambridge, UK

Correspondence to Vasilis Kosmoliaptsis, Department of Surgery, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ, UK. Tel: +44 1223 761 337; fax: +44 1223 962 523; e-mail: vk256@cam.ac.uk

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KEY POINTS

- Assessment of donor HLA immunogenicity helps offset the risk of alloantibody production, following organ transplantation.
- Advances in computational molecular modelling techniques enable assessment of HLA-alloantibody interactions at the atomic level.
- HLA surface electrostatic properties are key determinants of HLA antigenicity (reactivity with specific antibody) and immunogenicity (ability to induce an antibody response).

responses. The challenge in clinical transplantation is to predict in advance (and thereafter avoid) donor HLA mismatches that are most likely to cause a deleterious immune response in a particular recipient. In recent years, assessment of donor HLA immunogenicity has focused on the description and characterization of epitopes expressed on the surface of HLA molecules that are recognized by recipient B-cell receptors and lead to the development of alloantibody responses. In this article, we review studies investigating the molecular basis of antibody–antigen interactions and present computational approaches to determine the complex physiochemical and structural properties of B-cell epitopes. We also provide examples of how computational molecular modelling techniques may be applied to analyse the differential functional effect of alloantibody binding to target HLA epitopes. Assessment of HLA antigenicity and immunogenicity at the atomic resolution level represents a promising area of future research that may enable a better definition of donor-recipient HLA compatibility and inform future organ allocation policy to maximize the benefits of transplantation.

PREDICTION OF HUMAN LEUKOCYTE ANTIGEN IMMUNOGENICITY

The degree of HLA disparity between a given donor–recipient pair is often expressed as an HLA mismatch grade in which the number of incompatible donor antigens at each locus (HLA-A, -B and -DR) is counted. A major limitation of this approach is that, within a given locus, mismatches are assigned equal importance regardless of structural or amino acid sequence differences between donor and recipient HLA type. It has been long recognized that mismatched donor HLA specificities vary widely in immunogenicity from those with potential to cause graft rejection to those which are apparently nonimmunogenic and have been designated as permissible (acceptable) mismatches [7]. Early attempts to predict the immunogenicity of HLA alloantigens were largely unsuccessful. In recent years, however, amino acid sequence analysis of HLA molecules and structural information obtained from X-ray crystallography have enabled comparison of donor and recipient HLA type at the molecular level, providing an opportunity for a more rational approach to the study of HLA immunogenicity. Most significant in this regard has been the introduction of HLAMatchmaker, a computer algorithm that examines HLA compatibility based on a linear amino acid sequence (triplets) and more recently structural (eplets) definition of immunogenic B-cell epitopes [8,9]. Assessment of the immunogenic potential of a mismatched alloantigen is based on intralocus and interlocus comparisons of its epitope repertoire with that of the recipient HLA molecules. Donor alloantigens with unique combinations of epitopes are considered highly immunogenic and predicted to generate strong alloantibody responses. HLAMatchmaker has been used with success to analyse HLA-specific alloantibody reactivity in sera with complex alloantibody profiles [10–12] and to identify acceptable HLA mismatches for highly sensitized patients [13,14]; HLA class I mismatched kidney transplants with a low number of HLAMatchmaker-defined epitope (eplet) mismatches were shown to have graft survival times comparable to those of HLA class I matched transplants [15] (HLAMatchmaker is reviewed in [16,17]). Significantly, Wiebe *et al.* [18▪▪] have recently shown that application of an epitope (eplet)-based matching approach better predicts de-novo HLA class II alloantibody development, following kidney transplantation compared with conventional serologically-based HLA matching.

Evidence provided by studies applying the HLAMatchmaker concept suggests that epitope-based HLA matching may enable an improved assessment of donor–recipient HLA compatibility. It is, however, evident that the presence of a mismatched epitope on a donor HLA alloantigen does not always lead to a recipient alloantibody response. The immunogenicity of a given HLA epitope may be inferred by the frequency of epitope-specific alloantibody responses following exposure of renal transplant patients to a particular HLA mismatch [19]. Moreover, the immunogenicity of an HLA epitope may be predicted on the basis of the physiochemical properties of the polymorphic amino acids that constitute the epitope. Analyses of HLA class I and class II alloantibody responses in highly sensitized patients awaiting kidney transplantation demonstrated that the capacity of an HLA alloantigen to induce an alloantibody response depends not only

on the number of its amino acid polymorphisms compared with recipient HLA molecules, but also on the physiochemical disparities (hydrophobicity and electrostatic charge) between mismatched amino acids [20,21]. A retrospective analysis of HLA-A, -B and -DR matched or single HLA-A or -B mismatched deceased donor renal transplants ($n = 5247$) undertaken in the United Kingdom (1990–2005) showed that this approach may allow selection of low-immunogenicity donor-recipient HLA combinations with a beneficial effect on graft outcomes [22].

B-CELL EPITOPE STRUCTURE

Current approaches aimed at determining HLA immunogenicity rely heavily on amino sequence information that provides a limited insight into the tertiary characteristics of B-cell epitopes. Epitopes that form antigenic determinants can be viewed as three-dimensional arrangements of surface-accessible amino acid residues that form noncovalent interactions with the antigen-binding site of the antibody (paratope), resulting in a specific and high-affinity interaction. Antibodies are all-beta proteins that consist of two identical heavy and light chains dimerized by a disulphide bond. They have a highly conserved sequence, and therefore structure, with the exception of the fragment antigen-binding (Fab) region, which forms the paratope from a subset of residues in the six complementarity determining region (CDR) loops. The antibody paratope binds to any one of a small number of structurally similar epitopes with varying degrees of affinity. A B-cell epitope may be either linear or conformational; linear epitopes are created by sequential residues of the polypeptide chain whereas conformational epitopes that are far more prevalent, are formed by nonconsecutive residues that are colocated upon protein folding. This direct recognition of native conformational epitopes by surface immunoglobulin antigen receptors on B-cells is in contrast to self-restricted T-cell antigen receptor recognition of proteolytically-cleaved antigenic peptides presented in the peptide-binding groove of HLA molecules.

The gold standard for epitope identification is the structural determination of the antibody–antigen complex using techniques such as X-ray crystallography. This allows precise identification of interface residues and their bonding interactions. Although approximately 1800 immunoglobulin structures have been deposited in the Protein Data Bank [23], relatively few antibody–antigen complexes, and only one human HLA–antibody complex, have been determined [24]. The antibody–antigen interface usually comprises 15–22 residues that form hydrophobic, van der Waals, hydrogen bonds and salt bridges [25,26▪]. These residues contribute most of the energy of binding and are often located in the centre of the interface [27]. Although relative success has been achieved in T-cell epitope prediction [28–30], B-cell epitope prediction has been less successful; this is in part due to the requirement for structural information but also because residues that comprise an immunogenic B-cell epitope are difficult to distinguish from those that are not immunogenic [31▪]. When protein or antibody structures have not been crystallographically determined, sophisticated computational techniques for predicting molecular structures are available to assist with determination of B-cell epitopes. Most notable among these techniques is homology modelling, which is based on the principle of structure conservation among proteins with high-sequence homology [32,33▪]. Because the Fab region of the antibody is highly polymorphic, *ab-initio* methods have also been developed to more accurately predict the structure of the antibody paratope [34]. With antibody and antigen structures available, computational docking methods are employed to orientate the predicted antibody structure on the antigen followed by CDR optimization [34,35]. However, reliability remains a concern when predictions from computational docking methods are compared to crystallographically- determined antibody–antigen structures [36].

WHY HUMAN LEUKOCYTE ANTIGEN ELECTROSTATIC PROPERTIES ARE IMPORTANT?

Proteins are highly charged molecules due to the ionized or polar nature of 11 of the 20 amino acids at physiological pH. Protein electrostatic properties are determined by the number and distribution of such residues and their ability to form specific bonding interactions. Electrostatic forces are a source of long-range ($1/r^2$, where r is the distance from the charge) interactions that can attract two biomolecules to the point that short-range interactions, such as van der Waals forces, hydrogen bonds and salt-bridges, can stabilize the interaction [37]. Although hydrophobicity is crucial in protein folding, it does not represent a discreet force, but is better understood as an inability to create electrostatically-driven hydrogen bonds [38]. Hydrophobicity is, therefore, also considered when examining protein electrostatics. Many computational and experimental studies have established the relationship between protein electrostatic properties and function (reviewed in [39–41]). Electrostatic forces are particularly important determinants of the specificity and

selectivity of antibody binding [42–44]. High-affinity antibody–antigen binding is mediated through short-range electrostatic interactions [41]. Importantly, the process of antibody affinity maturation appears to involve the optimization of the electrostatic interactions in the antibody–antigen binding site [45]. In recent years, an increasing interest in the role of electrostatic properties in protein function has given rise to the concept of electrostatic motifs on the surface of biological macromolecules; an electrostatic motif represents a distinct topographical pattern of electrostatic potential in a three-dimensional space, which in the context of structurally-related macromolecules can help identify conserved regions that share a common biological function [46].

The apparent simplicity and elegance of the equations used to describe the fundamentals of classical electrostatic theory may mask the considerable difficulties involved in their application to complex systems such as macromolecules. Accurate modelling of such systems should account for the irregular molecular surface of biomolecules, complex interactions with polar water and mobile ions in the solvent, conformational flexibility and variation in side-chain protonation state [47,48]. Evaluation of protein electrostatic potential also requires knowledge of the protein structure at atomic resolution that can either be determined experimentally, most commonly using X-ray crystallography, or through computational prediction techniques such as homology modelling. From protein structure and parameters taken from a molecular mechanics force field, a detailed description of the distribution and nature of solute charges and, therefore, the electrostatic potential of a biomolecule can be calculated.

The complex shape of biomolecules renders analytical solutions of their electrostatic interactions in a solute–solvent system impractical. Therefore, numerical solutions are necessary that, in the broadest terms, can be achieved using either microscopic models or macroscopic (continuum) models [49]. Microscopic models use an all-atom, explicit, representation of solvent and solute to precisely evaluate the interaction of all components within the system. Because of the high number of possible conformations that must be sampled (high degrees of freedom of solute and solvent), microscopic modelling is computationally intensive and the results are often difficult to reproduce [50]. Much interest has, therefore, centred on macroscopic models that represent the solute as an area of fixed charge distribution with a uniform, low, dielectric constant... dielectric constant to account for the biomolecule's conformational flexibility, induced dipoles and titratable side-chains [51].

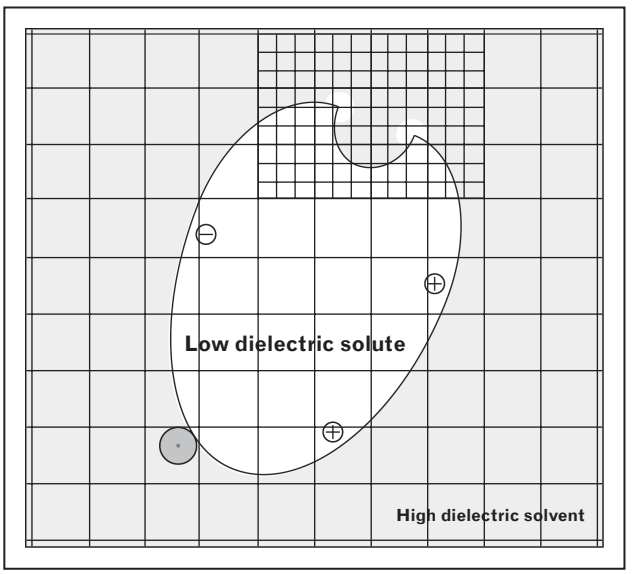

FIGURE 1. Schematic diagram of macroscopic evaluation of protein electrostatic potential. Macroscopic models treat the polarizability of the solvent as a high dielectric region, shown in light grey, and of the biomolecule as a low dielectric region, shown in white. The dielectric boundary between the solvent and the biomolecule (solvent accessible surface) is shown as a white line contour, which is derived by the centre of a rolling sphere with a radius of a water molecule (shown in dark grey) on the van der Waals surface of the biomolecule. Fixed atomic charges in the biomolecule are treated explicitly. A schematic representation of a three-dimensional grid on which the Poisson–Boltzmann equation is solved is shown in black. Focusing methods, in which the resolution of the grid is increased, are commonly used to give greater precision in the area of interest, such as a ligand-binding site.

The solute is surrounded by a high-dielectric medium to account for the high degrees of freedom of water (Fig. 1). The biomolecule–solvent boundary is defined by a rolling spherical probe, usually the size of a water molecule, traced over the van der Waals surface of the biomolecule [52,53]. Electrostatic potential of a biomolecule-solvent system is commonly calculated, numerically, using the Poisson–Boltzmann equation (PBE) [54,55▪▪]. The PBE incorporates Coulomb's law, Boltzmann's distribution of charged ions in response to protein charges and the dielectric constants of solvent and solute [51]. For solving the PBE, the macromolecule and a region of the surrounding solvent are mapped onto a three-dimensional Cartesian lattice such that each lattice point represents an atomic-scale region region of either the molecule or the solvent (Fig. 1) [47,49]. At each point, values are assigned for the charge density, dielectric constant and ionic strength parameters of the PBE. PBE solutions for the total electrostatic potential of a biomolecule–

solvent system have been validated using analytical solutions, microscopic models [56] and by experiment [57]. The application of continuum electrostatics theory to complex systems and the accurate prediction of protein structure have enabled qualitative and quantitative evaluation of the electrostatic properties of biomolecules that has found many applications in research of complex biological phenomena [58–60].

ELECTROSTATIC PROPERTIES OF HUMAN LEUKOCYTE ANTIGEN MOLECULES: INFERENCE ON HUMAN LEUKOCYTE ANTIGEN IMMUNOGENICITY AND ANTIGENICITY

The availability of high-quality, experimentally-resolved HLA structures combined with advances in comparative structure modelling and in the application of electrostatics theory in the study of

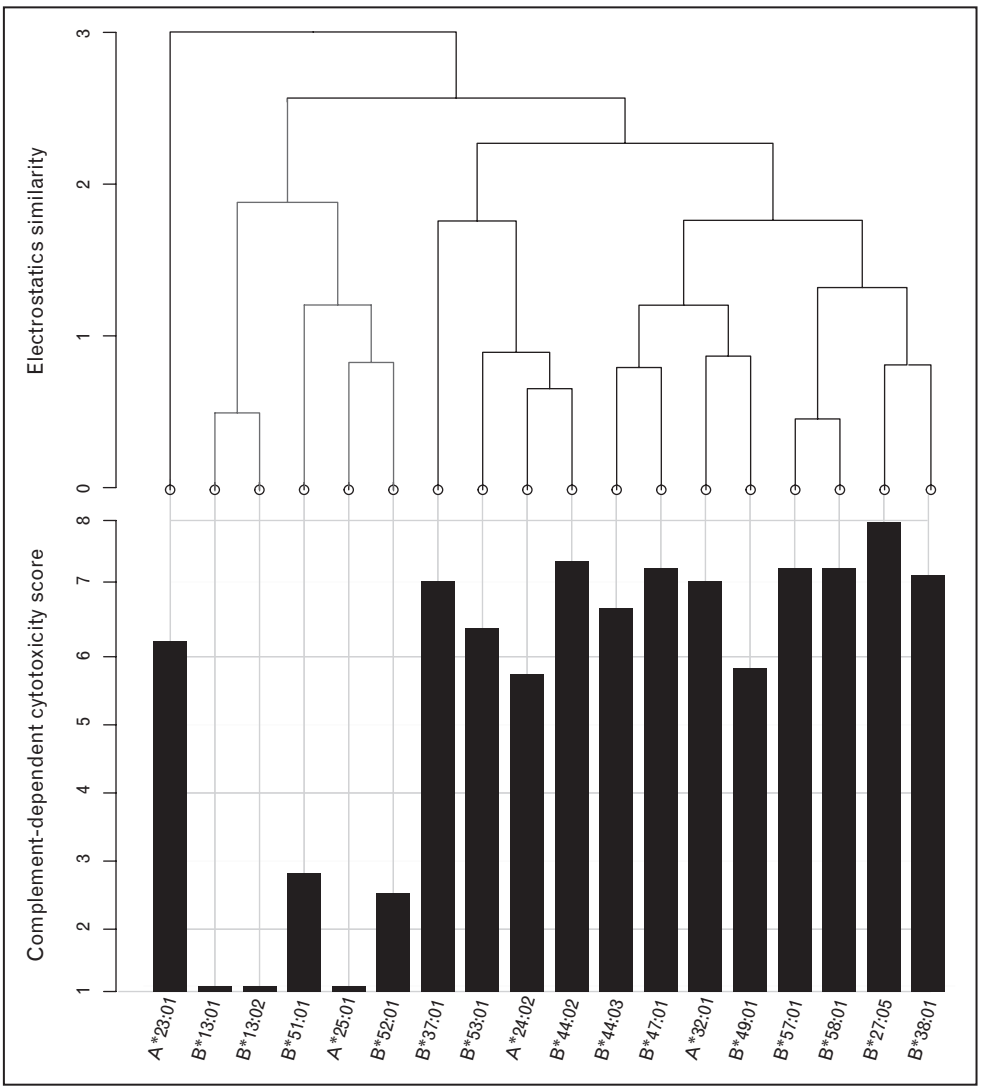

FIGURE 2. Association of Bw4 epitope surface electrostatic potential with cytotoxic reactivity of a Bw4-specific human monoclonal antibody. The electrostatic characteristics over a region, or epitope, of interest can be quantitatively compared using the Hodgkin index [66–68]. To avoid interference from small variations in structure, the field of comparison is a 'skin' raised 3 Å from the molecular surface. A pair-wise, all-versus-all, comparison creates a distance matrix, which can be represented as a dendrogram that clusters alleles that are electrostatically similar and separates those that are electrostatically disparate. Duquesnoy *et al.* [64] showed that VDK8F7 is a Bw4-specific human monoclonal antibody that binds to multiple Bw4-expressing alleles. Luminex C1q and lymphocytotoxicity assays revealed no reactivity of VDK8F7 with HLA-A*25:01, -B*13:01, -B*13:02, -B*51:01 and -B*52:01 despite expression of the Bw4-canonical motif. Quantitative comparison of the surface electrostatic properties of the Bw4 epitope expressed by the alleles shown revealed that C1q and lymphocytotoxicity negative alleles possess Bw4 epitopes with distinct electrostatic profile (highlighted in grey) compared with Bw4 epitopes on lymphocytotoxicity positive HLA alleles.

macromolecules provides a unique opportunity to increase our understanding of the relationship between structure and function in the context of HLA antigenicity and immunogenicity. A recent study demonstrated that the structural and physiochemical composition of the commonly expressed HLA Bw4 and Bw6 epitopes is characterized by distinct topographic patterns of electrostatic potential in three-dimensional space (electrostatic motifs) that are conserved among HLA molecules. This study suggested that conservation of the epitope electrostatic motif, despite differences at the amino acid sequence level, may account for observed patterns of serologic cross-reactivity [61]. In support of the link between HLA antigenicity and electrostatic properties, substitutions of critical amino acids leading to abrogation of antibody binding to a Bw6 epitope were reflected at the structural level by dramatic changes of the epitope's electrostatic pattern; conversely, mutation of residues noncritical for antibody reactivity had minimal structural and electrostatic effects [61].

The introduction of highly-sensitive laboratory assays has enabled detection, in patient sera, of an array of alloantibodies with variable HLA-binding affinities whose clinical significance remains elusive [62,63]. Duquesnoy *et al.* [64] have recently examined patterns of reactivity of human cytotoxic HLA class I epitope-specific monoclonal antibodies in immunoglobulin G (IgG)-binding and complement component C1q-binding Luminex assays in comparison with complement-dependent lymphocytotoxicity data reported at the 13th International HLA Workshop. They showed that the Bw4-specific monoclonal antibody VDK8F7 reacted with most Bw4-carrying alleles in Luminex IgG-binding assays, except HLA-A*25:01, HLA-B*13:01 and HLA-B*13:02 [64]. Interestingly, although VDK8F7 reactivity against the Bw4-carrying alleles HLA-B*51:01 and -B*52:01 was demonstrated on the Luminex IgG-binding assay, antibody binding was not associated with C1q engagement nor with cytotoxicity against HLA-B*51:01 and -B*52:01 expressing lymphocyte panels [64]. To further dissect these observations, we have generated the three-dimensional structures of the above HLA class I alleles using MODELLER [32] and calculated their electrostatic potential using the advanced Poisson–Boltzmann solver [65]. Figure 2 shows a quantitative, pairwise all-versus-all, comparison of the Bw4 epitope surface electrostatic potential for the relevant Bw4-expressing HLA molecules. These comparisons generate a distance matrix that can be represented as a dendrogram that clusters electrostatically similar epitopes and separates those that are electrostatically disparate. Remarkably, HLA-A*25:01, -B*13:01 and -B*13:02 (Luminex IgG, C1q and lymphocytotoxicity negative with VDK8F7 antibody) and HLA-B*51:01 and -B*52:01 (C1q and lymphocytotoxicity negative with VDK8F7 antibody) express Bw4 epitopes with similar surface electrostatic potential that is quantitatively discrete from the surface electrostatic potential of the Bw4 epitopes expressed by Luminex IgG and lymphocytotoxicity positive alleles (Fig. 2 [66–68]). These findings suggest that the electrostatic properties of HLA epitopes may be intrinsically linked with the affinity of alloantibody-HLA binding and the differential functional effect of their interaction (e.g., ability to bind complement), providing new insights into the molecular basis of HLA antigenicity.

CONCLUSION

Recognition of donor HLA alloantigens by the recipient immune system leads to the induction of HLA-specific antibodies that are increasingly being recognized as critical contributors in the pathogenesis of acute and chronic allograft damage. Donor and recipient HLA matching helps offset the risk of humoral alloimmunity, but current assessment of HLA compatibility is inadequate. It is now well established that the potential of donor HLA to generate humoral immune responses is not only an intrinsic property of the mismatched alloantigen but also depends on the HLA phenotype of the recipient. Information obtained by analyses of polymorphic amino acids at continuous and discontinuous HLA sequence positions is useful for predicting the relative immunogenicity of individual HLA mismatches; however, sequence information alone provides limited insights into key determinants of B-cell epitope immunogenicity, such as the orientation, accessibility and physiochemical properties of amino acid side chains. In recent years, structural information from X-ray crystallography and advances in computational molecular modelling techniques have enabled structural comparison of donor and recipient HLA type, providing an opportunity for a more rational approach for determining HLA compatibility. Assessment of the surface electrostatic properties of HLA B-cell epitopes and computational analyses of HLA-alloantibody interactions represent a promising area for future research into the molecular basis of HLA immunogenicity and antigenicity.

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Conflicts of interest

The authors declare no conflicts of interest.

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