Epitope-based human leukocyte antigen matching for transplantation: a personal perspective of its future

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Purpose of review
This study reflects my personal experience with the characterization of human leukocyte antigen (HLA) epitopes and their significance in HLA matching for transplantation. It offers a subjective assessment what further studies are needed to have this concept be applied in the clinical setting.

Recent findings
This study addresses the structural characteristics of antibody-reactive HLA epitopes determined by different methods, eplet-associated antibody analysis and acceptable mismatching for sensitized patients and eplet immunogenicity and determination of mismatch permissibility.

Basic implications
for clinical practice and research consider the need for further studies of the structural basis of antibody-verified HLA epitopes determined in different techniques and their clinical relevance, the biological basis of epitope immunogenicity and determinations of permissible mismatches and a computerized clinical transplant database with an Artificial Intelligence component that can generate evidence-based information for the practical application of epitope-based HLA matching.

Keywords
acceptable mismatch, eplet, human leukocyte antigen antibody, human leukocyte antigen epitope, HLAMatchmaker, permissible mismatch, structural epitope

INTRODUCTION

Human leukocyte antigen (HLA) antibodies are important mediators of allograft rejection leading to transplant failure. Such antibodies can develop after exposure to HLA mismatches not only after a transplant but also during pregnancy and after blood transfusion. Traditionally, these antibodies have been interpreted as being specific for HLA antigens, but we know now that HLA antibodies recognize epitopes presented by mismatched alleles. During the years, I have written many review articles about structural descriptions of HLA epitopes, their specific recognition by HLA antibodies and how they are associated with allograft rejection and transplant failure [1–6,7▪,8▪▪]. The HLAMatchmaker website www.epitopes.net has numerous articles and epitope analysis programmes that can be downloaded.

Instead of writing yet another review, I am offering a perspective of the future of epitope-based HLA matching and what studies are needed to address important questions. At the time of publication of this paper, I will have celebrated my 80th birthday and I am reminded of the limited amount of time left to study my favourite scientific topic. Therefore, this article is intended as my subjective assessment of the role of HLA epitopes in transplantation and what studies are needed to advance this topic. This information might be useful to transplant professionals interested in the clinical application of this concept.

This study reflects my personal experience and opinion and is divided into three sections: structural characteristics of HLA epitopes recognized by alloantibodies, epitope-based acceptable HLA mismatching for sensitized patients and control of

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KEY POINTS

- HLA matching at the epitope level is the way to go in organ transplantation.
- Eplets are key components of epitope-based class I and class II HLA matching.
- Epitope analysis of serum HLA antibodies is the most efficient approach to identify transplant donors for sensitized patients.
- Permissible HLA mismatches with low eplet loads and avoidance of highly immunogenic eplets offer new opportunities for nonsensitized transplant candidates.

antibody responses to mismatched HLA epitopes. Although it incorporates recent observations by other investigators, this article is not intended as a comprehensive review of the current literature.

STRUCTURAL CHARACTERISTICS OF ANTIBODY-REACTIVE HUMAN LEUKOCYTE ANTIGEN EPITOPES

As described in previous publications, a characterization of HLA epitopes requires an understanding that antibodies bind to protein epitopes through six complementarity-determining region (CDR) loops, three of them H1, H2 and H3 are on the immunoglobulin heavy chain and L1, L2 and L3 are on the light chain. Each loop interacts with a small set of amino acid residues in the so-called structural epitope with 10–25 residues distributed over a surface area of about 700–900 $Å^2$.

The CDR-H3 loop binds to the so-called functional epitope centrally located within the structural epitope and which displays a dominant role in determining antibody specificity. From the surface area of a structural epitope, one can calculate that the other CDRs are within a 15 Å radius. The HLAMatchmaker algorithm considers so-called eplets to be equivalent to functional epitopes. Eplets are defined by amino acid configurations within a 3–3.5 Å radius of a polymorphic residue in an antibody-accessible location on the HLA molecule. Eplet descriptions use only polymorphic residues with standard one-letter codes in sequence locations on the molecular surface. For instance, the 127K eplet is defined by a lysine residue in sequence location 127; this eplet is shared between many A2, A23, A24, A68 and A69 alleles and 62GE is shared between A2, B57 and B58 alleles. Some eplets have subscripted numbers that indicate more than one residue configuration. For example, $52PQ_2$ has two configurations shared by the same group of DQ5 and DQ6 alleles, namely 52PQ and 85VG.

The International HLA Epitope Registry (http://epregistry.com.br) has records of current eplet repertoires for HLA-ABC, HLA -DRB, HLA -DQ, HLA -DP and MICA alleles. Eplets are theoretical considerations and we must raise the question which ones are recognized by specific alloantibodies induced by HLA mismatches. Indeed, many eplets have been verified experimentally with informative antibodies. For each locus, the Registry has a list of antibody-verified eplets and this information is periodically updated.

Single allele bead (SAB) binding assays are now commonly used to analyse eplet-associated antibody reactivities with HLA typed panels and there are several patterns indicated by mean fluorescence intensity (MFI) values. An antibody often reacts with alleles sharing the same eplet with the immunizing allele; such finding suggest that the specific epitope is equivalent to that eplet. Many specificities of sera used for traditional serological typing of HLA antigens correspond to distinct eplets [7▪]. Similarly, several so-called public determinants in cross-reacting groups (CREGs) have corresponding eplets, but HLAMatchmaker has identified many other antibody-verified eplets shared between groups of alleles that have not classified as CREGs. In other words, antibody-verified eplets offer more complete assessment of the current repertoire of HLA epitopes. However, there are other levels of complexity for HLA epitopes reacting with specific antibodies.

Certain eplet-associated antibodies react with other eplets surrounded by nearby residue differences (within 3 Å). For instance, the 82LR eplet, which corresponds to the serologically defined Bw4 epitope, has two closely nearby residues 80I and 80T. Many 80I82LR-induced antibodies react only with 80I82LR-carrying alleles; we call them specific for 80I. There are other antibodies that react also with 80T82LR-carrying alleles; their MFI values can vary between high and low when compared with the reactivity of 80I82LR alleles. Another example is the cross-reactivity between 163LS on B44, B45 and B82 alleles and 163LG on B*15 : 12; the epitope is called 163LS/G [9]. These reactivity patterns reflect the so-called Landsteiner type of serological cross-reactivity whereby different structurally related epitopes react with the same antibody [10].

All antibodies have multiple CDRs that bind to different amino acid configurations within a 15 Å radius inside a structural epitope. Immunology textbooks describe the importance of the so-called affinity maturation process during the antibody response, whereby DNA segments corresponding to CDRs undergo mutations that lead to increased binding by such CDRs to residues in structural

epitopes. HLAMatchmaker considers a centrally located CDR (likely CDR-H3) to bind specifically to a mismatched eplet, but the formation of the antibody-epitope complex involves other CDRs especially those that have undergone affinity maturation. Such CDRs bind to residue configurations elsewhere within the structural epitope. These residues might be the same for all eplet-carrying reactive alleles but, as we cannot determine what they are, we can only state that such antibodies are eplet-specific.

Antibody reactivities of eplet-carrying alleles are often affected by polymorphic residue differences with the immunizing allele. Such residues must reside within a 15 Å radius of the eplet and they can be identified with amino acid sequence comparisons. Accordingly, all reactive eplet-carrying alleles in the SAB panel share the polymorphic residue configuration, which has been referred to as a 'critical contact sites' for antibody binding [11], whereas eplet-carrying alleles with different residues are nonreactive (or less reactive) with antibody.

The HLA Epitope Registry has many examples of epitopes defined by eplets paired with other residue configurations; these so-called eplet pairs can be found on both class I and class II antigens. As an example, the antibody-verified 82LR eplet has different epitope variations described by eplet pairs such as 82LR + 90D, 82LR + 145RA, 82LR + 138K and others [12]. For some pairs, the critical contact site is in the same alpha-1 domain, whereas for others, it resides in the alpha-2 domain.

Most antibody-verified DRB epitopes are equivalent to eplets and a few correspond to eplet pairs [13,14]. This low number might reflect the fact that the monomorphic DRA does not have polymorphic residues that can define DRB-DRA eplet pairs. It should be noted that for some DRB epitopes, site mutagenesis of the DRA residues affects the reactivity with antibody [15].

On the contrary, DQ and DP heterodimers have both polymorphic alpha and beta chains. The reactivity of certain DQ and DP antibodies are affected by amino acid residue differences on both chains [16–20]. Such residues must be in sequence positions within a 15 Å radius, sufficient for contact by different CDRs of antibody.

Certain structural HLA eplets have within a 15 Å radius, residues that are part of the peptides bound to HLA molecules. Several studies have shown that the composition of such peptides affect the binding of mAbs to mismatched eplets [21–23]; such antibodies have been referred to as peptide-dependent. One might suspect that for certain antibodies, their reactivity might be affected by the bound peptide repertoires on SAB alleles but, there is currently no experimental evidence.

MORE STUDIES ARE NEEDED TO IDENTIFY ANTIBODY-VERIFIED HUMAN LEUKOCYTE ANTIGEN EPITOPES

At present, the repertoires of antibody-verified HLA epitopes must still be considered incomplete and more analyses of informative antibodies are needed especially in geographic regions with different population distributions of HLA alleles. Such studies require allele level HLA typing information about the immunizer and antibody producer and the use of informative SAB panels. Postpregnancy sera are good candidates for such studies because the antibody producer is exposed to only one immunizing paternal allele for each ABCDR locus and only one DQ and DP heterodimer. Posttransplant sera tend to give more complex reactivity patterns that need to be dissected with absorption-elution studies with informative SAB alleles, with single donor platelet preparations and/or B cells. Human mAbs generated from cloned transformed B cell lines will offer excellent opportunities; it has become possible to analyse supernatants of B cell cultures generated from peripheral blood of transplant recipients [24]. Binding kinetics and affinity constants can now also be determined for HLA-specific antibodies [25].

TECHNIQUE-DEPENDENT EPITOPE SPECIFICITIES OF HUMAN LEUKOCYTE ANTIGEN ANTIBODIES

The IgG-binding assay with SAB alleles is the most common test for HLA antibody analysis and commercial vendors have incorporated HLAMatchmaker for epitope specificity determination. The MFI value represents a measure to distinguish between positive and negative reactions of sera with HLA antibodies, but practice has shown often enough that interfering substances in sera and denaturation of HLA molecules on SAB alleles make interpretations difficult [26,27].

The C1q-binding and C3-binding assays with SAB panels represent additional approaches to HLA antibody analysis. Comparisons between the different binding assays are often difficult for sera with multiple HLA antibodies that compete with another, and as indicated above, other serum factors might also be involved.

These binding assay variations consider the concept that many HLA antibodies can activate the complement pathway that leads to inflammatory injury of the allograft. Antibody-epitope complex formation activates the C1q-receptor on Fc part of

the antibody molecule and initiates the antibody-bound C1qrs complex as the first step of the classical complement pathway leading to C3-binding and eventually cytotoxicity. These steps require free energy released upon the formation of the antibody-epitope complex in the amount must be sufficient for complement-dependent, antibody-induced mechanisms of allograft injury.

There are structural epitope-associated differences between antibody reactivities of HLA antibodies tested in IgG- and C1q-binding assays and complement-dependent cytotoxicity (CDC). We have studied purified human mAbs originating from pregnant women; each one had CDC reactivity with the immunizing allele [28]. All immunizer-specific eplet-carrying alleles in the SAB panel were reactive in IgG-binding assays; several of them reacted equally in C1q-binding and CDC tests with a large panel of lymphocytes. There are also monoclonals that react equally in IgG- and C1q-binding but only partially with a lymphocyte panel because certain eplet-carrying alleles are nonreactive in CDC. Other monoclonals have partial reactivity in both C1q-binding assays and CDC.

These technique-dependent reactivity patterns are associated with amino acid differences within the structural epitope corresponding to an eplet. Partially reactive alleles lack nearby critical residue configurations needed for binding with other CDRs of antibody and the subsequent activation of the complement pathway leading to cytotoxicity. These findings are consistent with the concept that each HLA antibody has a CDR specific for a given eplet and that there are additional CDRs that bind to residue configurations within a 15 Å radius. Each binding with a CDR contributes to the release of free energy upon antibody-epitope complex formation and the total amount affects the subsequent biological activity initiated by antibody such as the complement pathway [28].

EPLET-SPECIFIC ANTIBODY ANALYSIS AND ACCEPTABLE MISMATCHING FOR SENSITIZED PATIENTS

In the clinical setting, the primary purpose of serum HLA antibody analysis of sensitized transplant candidates is to identify potential donors with acceptable mismatches. HLAMatchmaker has antibody analysis programmes to determine eplets specifically recognized by patient's antibodies and any allele expressing such eplets including alleles that have not been tested in SAB assays, can be considered as unacceptable mismatches. Epitope-based acceptable mismatching was first applied by Eurotransplant [29,30] and transplant programmes in France, Australia and Canada have been funded to investigate this concept.

The clinical implementation of this strategy raises a few questions. Which serum screening method should be used and what are the criteria for defining positive reactions for epitope-specific antibodies? How complete is the antibody detection in patients still carrying a rejected transplant? Several studies have shown increased serum HLA antibody reactivity after allograft nephrectomy [31–34]. Moreover, how do we know which antibodies are clinically relevant as a risk factors for transplant rejection [35]? Although retransplant candidates have likely been exposed to multiple epitope mismatches, the antibody specificities are generally towards a limited number of epitopes [31]. This suggests that the patient has developed a nonresponsiveness (tolerance?) to previously encountered mismatched epitopes. Could alleles expressing such epitopes be preferred as acceptable mismatches?

Many transplant centres use desensitization methods such as plasmapheresis, intravenous immunoglobulin and bortezomib to remove HLA antibodies, but these efforts have often met limited success. HLAMatchmaker analyses of sera might reveal differences in epitope reactivity before and after treatment and this information opens new windows of opportunities to identify acceptable mismatches.

EPLET IMMUNOGENICITY AND DETERMINATIONS OF PERMISSIBLE HUMAN LEUKOCYTE ANTIGEN MISMATCHES

HLA matching at the epitope level also benefits transplant outcome in nonsensitized patients without donor-specific HLA antibodies before transplantation. HLAMatchmaker can convert a donor HLA type to an eplet load, that is the number of class I and class II mismatched eplets, and this depends on the HLA type of the recipient. Numerous studies with different organ transplants have shown associations between eplet loads with donor-specific HLA antibody responses, allograft rejection and transplant survival [8▪▪,36,37]. Wiebe and Nickerson [38▪] have shown that information about DR and DQ eplet loads permit personalized immunosuppression strategies aimed to control the alloimmune response and minimize risks for drug toxicity, infection and malignancy. At least two paediatric transplant programmes are now using donor selection strategies based on eplet loads [39–41].

The application of the eplet load concept is not enough for the strategy of permissible HLA mismatches aimed to achieve greater long-term

transplant success. Minimizing exposures to immunogenic eplets will be essential. The immunogenicity of eplets can be determined by empirical studies that determine the frequencies of eplet-specific antibody responses [9,42], but it is more important to understand the biological basis of eplet immunogenicity.

Antibody responses begin with the activation of B cells with specific immunoglobulin-like surface receptors that bind to epitopes on immunizing antigens. Interactions with helper T cells promote the proliferation and differentiation including affinity maturation and immunoglobulin class switching and, eventually, antibody producing plasma cells. The immunogenetic relationship between the antibody producer and immunizing allele affects the antibody response to a mismatched eplet. Three complimenting theories have been forwarded [43].

The nonself-self paradigm of HLA epitope immunogenicity emerged from observations that antibody-verified defined by eplet pairs always include a self-configuration present on one of the HLA alleles of the antibody producer [44–46]. It is based on the postulate that a group of B cells have with low-avidity immunoglobulin receptors for self HLA epitopes. Such B cells remain inactive until exposure to a nonself eplet, which can trigger a strong alloantibody response through interaction with CDR-H3, which has distinct physiochemical characteristics including residue sequence lengths. The nonself-self paradigm predicts that B cell activation by mismatched eplets can only occur if the remainder of the structural epitope of the immunizing allele has primarily self-residues present on one of the alleles of the antibody producer. This concept is consistent with data that antibody-verified eplets have structural epitopes consisting of self-residues [46].

The antigenicity and immunogenicity of HLA epitopes are also influenced physiochemical properties including electrostatic potentials of the HLA molecular surface [47–50]. A recent study has shown that DR and DQ molecular mismatches determined by eplet analysis, amino acid mismatching and electrostatic mismatches are all correlated with the development of donor specific antibodies [51].

B cell epitope prediction programmes have been used in the design of vaccines and the generation of specific antibodies used in immunotherapy and immunodiagnostics. Most of them have rather low accuracy rates. We have identified a promising program named ElliPro that can calculate protrusion scores for amino acid residues on antigenic proteins in three-dimensional complexes with antibodies [52]. ElliPro (available on the website-based Immune Epitope Database www.iedb.org) has been used to analyse the HLA-ABC eplet repertoire in the HLA epitope registry. Antibody-verified eplets and eplet pairs have amino acid residues with much higher ElliPro scores than eplets for which no specific antibodies have been found. Eplets with very low ElliPro scores might turn out to be nonepitopes incapable of inducing antibody responses.

Activated B cells need T-cell help for their antibody production and this involves indirect allorecognition by $CD4^+$ cells of peptides presented by B cells following the internalization and processing of Ig receptor-bound immunizing HLA allele. Spierings and colleagues have developed a computer program called PIRCHE (Predicted Indirectly ReCognizable HLA Epitopes) in which mismatched peptides (nonamers) in HLA allele sequences are predicted to bind with significant affinity to the grooves of class II (DRB1) recipient alleles [53,54]. Although PIRCHE-II numbers correlate with the development of donor-specific HLA antibodies after transplantation and pregnancy [55–58], it has been suggested that the PIRCHE effect on the HLA antibody response is complicated [59,60].

CONCLUSION

Altogether, clinical studies have demonstrated statistically significant associations between rejection-related transplant outcome and parameters such as eplet loads, molecular mismatches, PIRCHE numbers and physiochemical characteristics of HLA residue configurations. Statistical associations have limited practical value regarding the implementation of an epitope-based donor selection strategy, which must be effective for each patient and depends on the understanding how the donor-specific antibody response relates to recipient's HLA type and which antibodies are clinically relevant. This will need a large clinical transplant database that incorporates an Artificial Intelligence component to be used for a continuous analysis of donor–recipient immunogenetic relationships, posttransplant serum antibody reactivity patterns and immunological events affecting transplant outcome. Such computer system can generate evidence-based information showing how epitope-based HLA matching can be applied in the clinical transplant setting.

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Conflicts of interest

There are no conflicts of interest.

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