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Minireview

# HLA Epitopes as Viewed by Antibodies: What Is it All About?

**A. R. Tambur[1,*] and F. H. J. Claas[2]**

[1]*Transplant Immunology Laboratory, Comprehensive Transplant Center, Feinberg School of Medicine, Northwestern University, Chicago, IL*
[2]*Department of Immunohaematology and Blood Transfusion, Leiden University Medical Center, 2300 RC Leiden, the Netherlands*
[*]*Corresponding author: Anat R. Tambur, a-tambur@northwestern.edu*

**The need for new approaches to define HLA antibodies, in the context of organ transplantation, is intensely debated among HLA professionals. In this review, we sought to provide background and perspective to current understanding of the immunogenicity of HLA mismatches with respect to the humoral alloimmune response and the definition of B cell epitopes. Initial data suggest that epitope matching not only assists in defining better matches for the current transplant, but also minimizes the risk of developing *de novo* HLA-donor-specific-antibodies posttransplant. In other words, other than lowering the risk of current graft rejection, epitope matching is likely to lower overall future sensitization levels and thus increases the likelihood of finding a compatible donor when the need for a retransplantation arises. More detailed knowledge of epitopes makes it possible to investigate what constitutes permissible versus non-permissible HLA mismatches. The currently available evidence suggest that epitope matching is the most rational way to decrease the risk of HLA-linked transplant rejection. This review is aimed at stimulating further and more intense collaborative effort in this field.**

**Abbreviations: AM, Acceptable Mismatch; CREG, Cross Reactive Group; CTL, Cytotoxic T Lymphocytes; HLA, Human Leucocyte Antigen; MFI, Mean Fluorescent Intensity; PRA, Panel Reactive Antibody; TCR, T Cell Receptor**

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The field of histocompatibility testing has seen rapid and successive bouts of progress over a fairly short period of time. It has been fraught with multiple competing methodologies in early years, leading to independent discoveries of the same antigens by different investigators and thus generating multiple notation systems simultaneously (1–3). Even when methodologies have been streamlined and collaborative workshops were put in place, revealing the extreme polymorphic nature of the system led to sequential nomenclature systems in order to capture new advances and understanding of the HLA complex (http://hla.alleles.org/nomenclature/index.html). This chain of events naturally caused significant confusion, especially among ''non-tissue-typers.'' Interestingly, using today's knowledge, it is clear that many critically important observations had been made already 30–40 years ago. Yet, full interpretation and implementation of that knowledge has only recently become possible.

The most widely used method to detect HLA antigens, and later HLA antibodies, was the complement dependent lymphocyte cytotoxicity assay (4). Soon it became clear that a significant cross reactivity between HLA antigens exists. That is, immunization against one HLA antigen can lead to the generation of antibodies towards additional, seemingly unrelated, antigens (i.e. sensitization against A2 may lead to antibodies recognizing also B57 and B58). Moreover, the seemingly unrelated antigen can be used to remove antibodies reactive with the original immunizing antigen, as has been shown by absorption/elution studies. These experiments, together with family studies, substantiated the theory that the HLA system is encoded by multiple genetic loci as an autosomal system of great complexity. The puzzling nature of the observed cross-reactivity was a source for numerous studies demonstrating that alloantibodies, as well as monoclonal antibodies, can recognize ''determinants'' that are shared by different HLA antigens (5) and that serologically defined HLA antigens can each express several allo-epitopes (6,7). In fact, this data served as the basis for the Cross Reactive Group—CREG—system annotation that was promoted and debated for many years as an approach for antibody identification and organ allocation matching (8).

In the early 1990s, many histocompatibility laboratories acquired the ability to type for HLA by molecular techniques. The innovation of HLA typing with higher accuracy and higher resolution demonstrated that many HLA-antigens are in fact ''families'' of different alleles, though the differences between these alleles were too minor to be recognized by serologic typing. The vast polymorphism of the HLA system was identified quickly, allowing better associations between HLA and disease susceptibility as well as explaining responses to different pathogens and correlations with

transplant rejection. Yet, although HLA molecules were implicated as targets for many immune responses, the nature of the interactions between HLA molecules and the effector arm of the immune system was not clear. A major breakthrough in conceptualization of the function of HLA molecules in health and disease came about with the elucidation of their three dimensional structure by crystallography and X-ray diffraction analysis. Bjorkman and colleagues revealed the symmetrical nature of the four extra-cellular-domain structure but more importantly, the clustering of most of the polymorphic amino acids at the top of the molecule, forming a large groove recognized to be the binding site for processed antigenic peptides (9,10). They and others continued to describe how minor modifications in the amino acid sequence between different alleles of the HLA system can create and alter the nature of the pockets within the antigen binding site. These changes are responsible for the allelic specificity in foreign antigen binding and its presentation (11). In the ensuing years, much research focused on predicting interactions between T cell receptors and HLA-peptide complexes. For example, one of the critical steps for effective antigen presentation and activation of the cellular arm of the immune response is proper processing of the antigenic source into peptides that can stably bind to the HLA molecule. One field in which such knowledge is critical is the design of peptides for vaccination. Several software algorithms are now available for peptide-epitope discovery such as MULTIPRED (12). Interestingly, a significant portion of HLA-peptide complexes do not elicit TCR response despite the fact that the complex seems to be stable. It is possible that some of these complexes are driving negative selection processes of the TCR, but it is also likely that specific interactions between the complementarity-determining regions (CDR1, 2, and 3) of the TCR variable domain contribute to effectiveness of TCR-HLA-peptide interactions. This, of course, adds an extra layer of complexity and emphasizes the difficulties in assigning peptide-epitopes or T cell-epitopes simply based on the linear sequence of amino-acids.

Similar to the TCR, antibodies also have complementarity-determining regions that make contact with multiple sites of the antigen (13). The critical work performed on peptide-epitopes and T cell-epitopes, together with ample data on HLA antigen amino-acid sequences and the preliminary data describing CREG epitopes, led to more detailed investigations of the interactions between HLA antibodies and their target epitopes on HLA antigens, termed here B cell epitopes, which is the focus of this review.

### *Identifying HLA antibody epitopes*

A critical component in our ability to study B cell epitopes is the innovation of analyzing antibody specificities using solid phase single antigen assays. The improved specificity and sensitivity of these assays and the increased breadth of reagents available for testing cumulatively provided new tools to appreciate the complexity and ambiguities of HLA antibody-antigen interactions. The availability of a large panel of human monoclonal antibodies has been particularly instrumental for the exact definition of B cell epitopes on the different HLA antigens. It soon became clear that every HLA antigen has a its own unique combination of epitopes, but at the same time, many of these epitopes are shared with some other HLA antigens (Figure 1 provides an example in which antigen 1 shares the ''circle epitope'' with antigen 2; the ''triangle epitope'' with antigens 3 and 4; the ''doughnut epitope'' with antigen 3; and the ''diamond epitope'' with antigens 2 and 3. Thus, while all 4 epitopes are expressed in more than one antigen, the unique combination of all 4 epitopes is present only in antigen 1). A counterpart to the technical advancement was the development of the conceptual, computer-based, algorithm by Rene Duquesnoy – HLAMatchmaker, focusing on the structural basis of HLA polymorphism. The original version of the HLAMatchmaker software considered HLA molecules as a linear sequence of amino acid triplets, representing motifs of potentially immunogenic epitopes. The matching portion of the software compares these 3-AA motifs between donor and recipient HLA molecules and determines the presence and number of mismatched triplets. The baseline hypothesis is that an amino acid polymorphism, shared between the recipient and the donor, will not be immunogenic and therefore cannot lead to the generation of antibodies. Thus, the software utilizes inter- and intra-locus subtraction of shared amino-acid sequences to identify potential immunogenic epitopes (14). The original software considered polymorphic triplets only in antibody-accessible sequence positions (quoting 132 polymorphic triplets in 40 sequence positions of the serologically defined HLA-A, -B, and -C antigens [15]). However, later versions use a more stereochemical modeling and refer to *eplets*, rather than restricting to triplets, as the core target of antibody recognition. These structural eplets are not limited to linear sequences and are

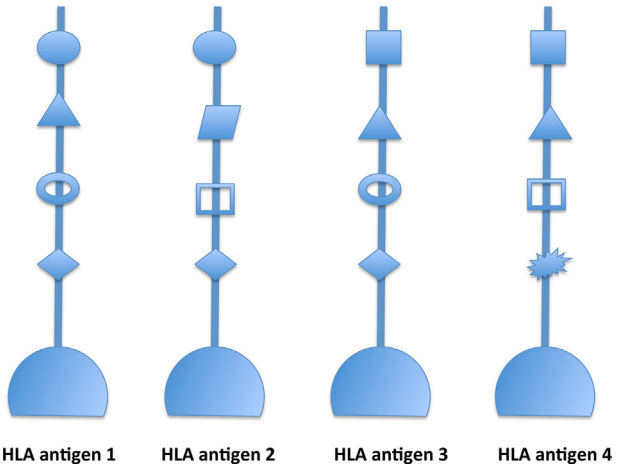


**Figure 1: An HLA antigen expresses a unique set of epitopes but the individual epitopes are often shared with other HLA alleles.**

now defined by a radius of 3 Å patches. Thus, in contrast to a triplet, an eplet can contain amino acids that linearly are discontinuous but are clustered together when observing the three-dimensional structure of the molecule (Figure 2 provides a depiction of the principle difference between a triplet and an eplet). The number of optional eplets has changed accordingly (16).

Paul Terasaki's group had taken a more hands-on approach. Their studies used absorption/elution methodologies in order to explain cross-reactions observed between different HLA antigens. Serum samples to be investigated were incubated with recombinant cell lines expressing multiple copies of a single HLA molecule; and then the antibodies binding to this particular HLA specificity were eluted. The eluates were then tested in the solid phase single antigen bead assay, demonstrating in most cases reactivity against not only the HLA molecule expressed by the recombinant cells, but also against additional HLA antigens that share one or a combination of amino acids (17). The target of these antibodies were then named TerEps and were described as the list of antigens that reacted with a given monoclonal antibody or alloserum. In later studies, Duquesnoy and Marrari demonstrated significant similarities between the two notation systems (18).

### *Correlation with clinical data*

With the understanding of the B cell epitope concept, the role of epitope load and its clinical implications were soon investigated. Lobashevski et al demonstrated a correlation between the number of amino acid residue mismatches and results of flow cytometry crossmatching in highly sensitized patients awaiting renal transplantation (19). Dankers et al (20) showed that the number of amino acid triplet differences between patient and donor is predictive of the antibody reactivity against the mismatched HLA antigens. Moreover, a study in a large cohort of patients who received HLA-mismatched kidneys, analyzing both the Eurotransplant and the United Network for Organ Sharing kidney transplant databases, demonstrated that the survival rates of grafts with a low triplet load were similar to the survival rate in recipients of zero HLA-A, -B mismatch kidneys (21). The logical progression from this point was to evaluate the use of this approach in alleviating the difficulties in finding compatible donors for the highly sensitized patients. Indeed, Duquesnoy and colleagues (22) evaluated the probability of finding a donor with 0–2 triplet mismatches for highly sensitized patients (PRA >85%) in a random population of donors. Using HLAMatchmaker, they showed that the chance of finding a compatible donor is much higher if one uses acceptable mismatches as a criterion for allocation rather than limiting matches based only on HLA-antigen identity with the donor. This new approach was soon adopted by the Eurotransplant as an additional tool to identify donors for highly sensitized patients in their Acceptable Mismatch (AM) Program and soon demonstrated decreased waiting time for a suitable kidney donor as well as excellent graft survival, comparable to that of nonsensitized patients (23). In this program, acceptable mismatches are defined as any antigen mismatch that is associated with a negative reaction in a cell-based PRA assay or by the use of the HLA matchmaker software.

The role of HLA epitope matching was recently demonstrated again in a group of kidney transplant recipients that were evaluated for *de novo* formation of HLA class II antibodies. In this study, Wiebe et al (24) showed that patients who developed HLA-DR or HLA-DQ *de novo* DSA had a higher number of locus-specific epitope mismatches. They also suggested an optimal threshold for epitope mismatching that lead to minimal or no DSA formation in their patient population, stating that epitope matching outperforms traditional low-resolution HLA antigen-based matching.

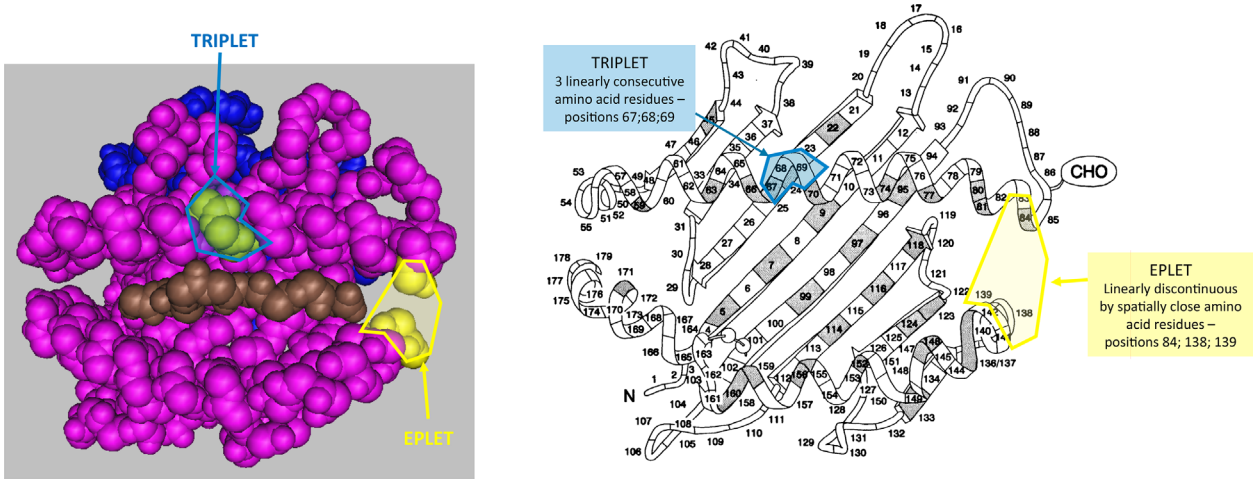


**Figure 2: Depiction of the principle difference between a triplet and an eplet.**

Analysis of antibody signatures from highly sensitized patients using the epitope concept allowed better understanding of antibody reactivity observed following a sensitizing event. For example, mismatched antigens can induce antibodies reactive not only with the immunizing antigen but also with other antigens that share epitopes with it. Thus, previously unexplained observations of non-donor-specific antibody formation posttransplantation, as mentioned earlier, can now have a scientific explanation. Numerous examples have been reported both for Class I antibodies (25) and for class II antibodies (26,27).

### *Immunogenicity and antigenicity*

The fact that immunogenicity of mismatched HLA antigens can vary from ''taboo'' to acceptable mismatches have been previously recognized and demonstrated (28,29). The critical question that remains, though, is what determines the antigenicity and immunogenicity of a certain HLA mismatch. It is well established that patients will develop HLA antibodies against a restricted number of mismatched epitopes (30); and that other patients will not develop HLA antibodies despite significant mismatching with their organ donors. One contributing factor is likely the HLA-class II phenotype of the recipient as it will influence the interaction between CD4+ T cells and B cells, which needs indirect recognition of donor derived peptides presented by HLA class II on the B cells (Figure 3). This phenomenon was initially reported by Fuller et al (31), and more recently by Otten et al (32) who demonstrated how the use of PIRCHE-II (predicted indirectly recognizable HLA epitopes, HLA class-II presented) can explain the generation, ornongeneration of *de novo* DSA in previously nonimmunized patients who received and lost their grafts in the face of similar mismatches. In addition, Duquesnoy postulated that significant similarity needs to exist between the HLA antigens of the antibody producer (patient) and those of the immunizing donor. To account for that, the concept of HLA epitope was extended to a *structural* epitope rather than the mere eplet differences between certain HLA sequences, leading to a nonself/self paradigm, requiring identity between most of the 15–25 surface amino acid residues that constitute the *functional* epitope, except for the implicated eplet. The functional epitope, in turn, is part of the structural epitope that comes in contact with the third complementarity-determining region (CRD) of the antibody (33,34). This hypothesis assumes the presence of low-affinity immunoglobulin receptors for self-HLA epitopes in the patients' circulation. Those immunoglobulins will not elicit an immune response against self-antigens, but once exposed to minor mismatches, a strong alloantibody response will ensue. In fact, overall B cell development and maturation relay on a similar process in which receptor editing following positive selection shapes the repertoire of alloreactive and autoreactive B cells (35). Indeed, much of the immune responses are directed at targets that deviate from self only by small modifications (36). Additional data to support Duquesnoy's hypothesis were recently presented by Tambur et al (37,38). The evolutionary logic to this phenomenon is that we evolved to attack self cells, infected

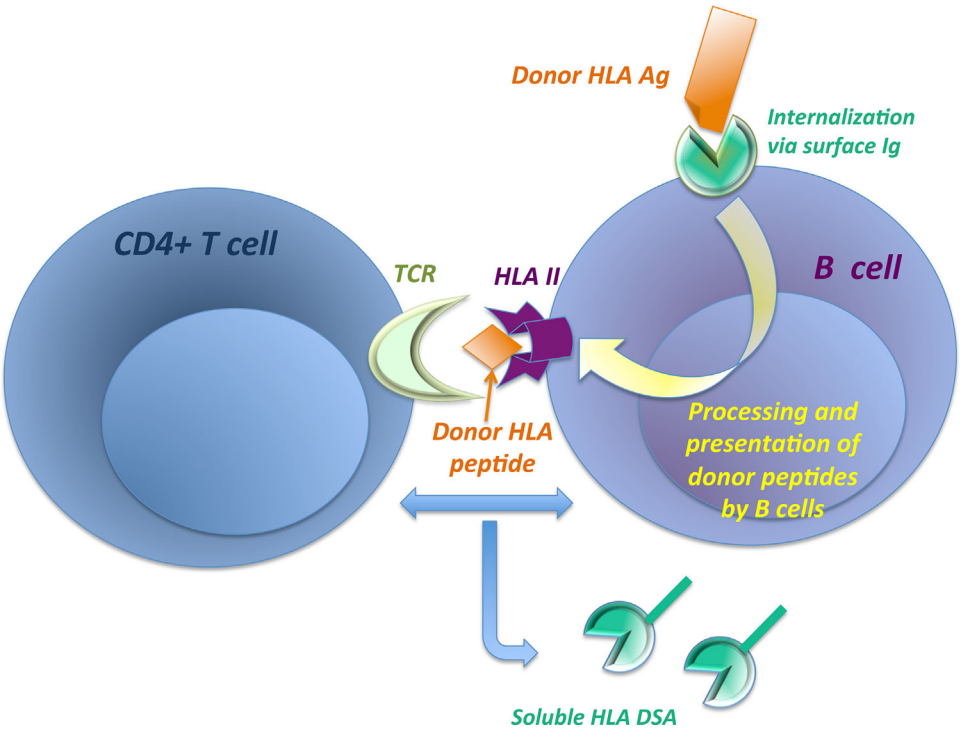


**Figure 3: Indirect presentation of HLA epitopes by HLA class II antigens.**

with intracellular parasites, on basis of peptides presented in the context of our own antigens.

The factors mentioned thus far are a likely explanation for the relative immunogenicity of a particular HLA-mismatch. Additional factors may determine the strength of these responses, whether measured as Mean Fluorescence Intensity (MFI), or actual titer of the antibody. Kosmoliaptsis et al (39) demonstrated that the number of continuous and discontinuous eplet mismatching is associated not only with the presence of alloantibodies but also with progressively stronger alloantibody responses. This information, though, as previously noted by other investigators (40), is not sufficient to explain the heterogeneity in binding strength, observed for well recognized alloantibodies such as antibodies to the public epitopes Bw4 and Bw6. By using comparative protein structure modeling and generation of high-resolution 3D structural and physiochemical models of common HLA class I alleles, Kosmoliaptsis and colleagues (41) were able to show differences in the number and distribution of polar and charged amino acid side chains outside of the conventional epitope site; changes which affected the folding and structural composition of the different HLA antigens expression Bw4 or Bw6 that were studies. This cutting edge technology provided new insight into the prediction and determination of class I HLA epitope antigenicity. The observation that antibody reactivity to a particular epitope can yield significantly different MFI values in a single antigen bead analysis is not unique to class I, as have been recently shown by Tambur and colleagues (42). Another factor that can affect the ability of antibody binding is the peptide presented within the HLA molecule and as a consequence tissue specific reactivity (43). While outside the scope of this mini-review, it is pertinent to mention that the technical limitations of the current solid phase assays, leading amongst others to a ''prozone effect'' or the detection of antibodies against denatured targets, may affect our ability to accurate assign B cell epitopes. These technical aspects, have been described in a comprehensive way in some recent review articles (44,45).

## Future Directions

Given the multitude of notation system changes that the HLA system endured over the past few decades, the authors recognize the less than enthusiastic response to any proposition of additional modifications. This should, however, be considered vis-à-vis the potential benefits to our patients' population. While we appreciate that additional data is required to fully define HLA epitopes as reflected by increasing number of epitopes registered at the dedicated website epregistry.ufpi.br, the time is ripe now for prospective studies moving beyond eplet matching and evaluating the advantages of ''B cell epitope matching'' in transplant outcome. For example, the most obvious population to benefit from such approach, as we have alluded to earlier (46) is the younger patients that will require more than one transplant in their lifetime. Common sense and initial data (24) infer that minimizing epitope mismatching will minimize their likelihood of developing *de novo* DSA. Given that many kidneys are currently lost due to chronic and antibody mediated rejections, the long-term impact of such prospect is very enticing and all efforts should be taken to investigate the utilization of epitope matching. Epitope analysis should also improve virtual crossmatching as it will allow the prediction of antigenicity of HLA alleles, which are not present in current single antigen reagent kits, based on epitope sharing with other alleles that are represented in the tested panel. Unfortunately, the data currently gathered by UNOS and other transplant organizations does not permit thorough investigation into the merit of the epitope matching. Obtaining high-resolution typing records of donor and recipient HLA antigens (including the six major loci – HLA-A, -B, -C, -DR, -DQ, and DP) will provide much of the required information. Should this biologically rational and promising approach be abandoned just because additional multicenter trials are required to substantiate its premise? Collaborative studies between the major transplant organizations, around the globe, are needed for the benefit of our patients.

Originally, HLA matching was intended to improve transplant outcome, therefore landing additional points for patients who were HLA-A, -B, and -DR matched with a specific donor. The scarcity of organs and geographic limitations caused a decrease in the perceived role of HLA matching to the point in which only HLA-DR matching (albeit at the antigen level, which does not necessarily indicate true matching) provides the patient with additional points. This artificially limited criterion for ''matching'' was put forth to attempt a preferential treatment of some populations, with questionable results on transplant outcome or rejection rates. The argument of impracticality of HLA-matching will be lessened with the use of epitope matching strategies, as there are less epitopes than HLA alleles. From the patients' perspective – a chance of improving outcome justifies every effort to collect the additional data.

## Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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