

CHAPTER 53

HLA Matching at the Epitope Level: The Way to Go

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INTRODUCTION

Among the many contributions Paul Terasaki has made to the human leukocyte antigen (HLA) field and transplantation, two are relevant to this chapter. First, he proposed in 2003 his original “Humoral Theory of Transplantation” (1), which led to extensive studies demonstrating that HLA antibodies cause allograft rejection and organ transplant failure (1, 2). Second, he reported more than 25 years ago that HLA antibodies recognize epitopes that can be defined by amino acid residues (3, 4) and pointed out the importance of HLA matching at the epitope level (5-7). Another activity was forwarding the concept of permissible mismatching to improve long-term graft survival (8, 9).

The traditional analysis of HLA antibody specificity has been based on the reactivity with HLA antigens such as anti-A1, anti-B7, and anti-DR1, but it is now recognized that such antibodies detect epitopes on HLA antigens. My research during the past ten years has focused on the structural characterization of epitopes on HLA antigens, how epitopes react with HLA antibodies (i.e. antigenicity) and how epitopes induce specific antibodies (i.e. immunogenicity). Epitope-based matching is important, not only for identifying acceptable mismatches for sensitized patients, but also for a better understanding of the sensitization process induced by an HLA mismatch. The latter might lead to new strategies to identify permissible mismatches so we can increase the success rates of transplants in non-sensitized patients.

Structural concepts of HLA epitopes

HLA epitopes can be described structurally by molecular modeling and amino acid sequence differences between antibody-reactive and nonreactive alleles. Each HLA antigen can be viewed as a collection of epitopes with polymorphic amino acid descriptions that must adhere to the general concepts of how antibodies interact with protein epitopes.

The antibody-combining site has three heavy chain and three light chain Complementarity Determining Region (CDR) loops that make contact with so-called structural epitopes comprising multiple amino acid residues distributed over a surface area of 700-900 square Ångstroms (10-13). A structural epitope has about 15-25 contact residues and within it lies the so-called functional epitope consisting of a few residues that play a major role in the binding with the centrally located third CDR-H3, which plays a dominant role in determining antibody specificity (14-17). The HLA Matchmaker algorithm considers an eplet to represent a functional epitope and that within the context of a structural epitope, other CDR-contacting residues on the HLA molecular surface must be within a radius of about 15 Ångstroms of an eplet (18). Eplets are defined by residue configurations within 3 Ångstroms of polymorphic residues on the molecular surface and they are considered key elements of epitopes that elicit specific antibodies. Their annotations are based on amino acid sequence positions and residue descriptions; examples are 62GE (shared between HLA-A2 and HLA-B17), 145L (on B13), 4Q (shared

between DR7, DR9, and DR53) and 52PR (shared between DQ4, DQ5, and DQ6).

Mismatched eplets can induce specific antibodies that react with all eplet-carrying HLA alleles, whereas the other alleles are nonreactive. Other antibodies recognize eplets paired with certain amino acid configurations generally within a radius of about 15 Ångstroms (19, 20). Interestingly, these pairs largely involve self configurations present in the HLA type of the antibody producer. We have also noted that residue differences within eplet-defined structural epitopes can explain technique-dependent variations in antibody reactivity (21). Differences in reactivity patterns of certain human cytotoxic HLA class I epitope-specific monoclonal antibodies in immunoglobulin-binding, C1q-binding Luminex assays, and complement-dependent lymphocytotoxicity assays are associated with distinct residue differences within 15 Ångstroms of specifically recognized eplets. These findings can be viewed in context of the release of free energy upon complex formation between epitope and the CDRs of antibody, which is necessary for binding stabilization and to induce conformational changes in the antibody molecule leading to complement activation.

Terasaki's group has done extensive analyses of antibodies tested in Luminex assays with recombinant HLA single antigen beads. These assays were done with mouse monoclonal antibodies against HLA and anti-HLA alloantibody samples from multiparous women, placentas, or patients sensitized by blood transfusions or organ transplants, and they yielded thus far almost 200 amino acid defined HLA class I and class II epitopes (22-26). Comparative analyses has shown that many of these epitopes correspond to HLAMatchmaker defined eplets (27, 28).

Under the auspices of the 16th International Histocompatibility and Immunogenetics Workshop, we have developed a website (<http://www.epregistry.ufpi.br>) for the International Registry of Antibody-Defined HLA Epitopes (29). Its goal is to develop a repertoire of HLA epitopes that have been verified by specific antibodies. Recent reports describe our progress so far on HLA-ABC,

HLA-DRDQDP, and MICA epitopes (30-32). The www.HLAMatchmaker.net website is another resource and has Excel based HLA epitope matching and antibody analysis programs that can be downloaded free of charge.

Epitope-based analysis of HLA antibodies in patient sera

Several HLAMatchmaker programs address the analysis of serum reactivity patterns for epitope-specific antibodies. The www.HLAMatchmaker.net website has Microsoft Excel programs and the Teresina group in Brazil has developed the user-friendly EpHLA software designed to analyze Luminex (33, 34). Antibody analysis programs by commercial vendors of Luminex HLA antibody kits also consider epitopes.

To optimize assignments of epitope specificities of antibodies, it is important to incorporate the patient's HLA type (preferably at the four-digit allele level) because this provides information about self eplets that cannot induce antibodies. HLA types of immunizers (for instance, a previous transplant) will identify the mismatched epitopes to which the patient has been exposed. This facilitates the interpretation of antibodies specific for donor-specific epitopes, including those that would be shared with non-donor alleles. Third-party epitopes on reactive alleles would be irrelevant unless there was another sensitization event. Although commercial assay kits have selected allele panels, HLAMatchmaker can also identify antibody-reactive eplets on other alleles not included in these kits.

The application of epitope-based analysis of antibody reactivity may also increase our understanding of otherwise unexplained sensitization patterns induced by a given HLA mismatch. For instance, we have recently reported a case whereby a transplant recipient who typed as B*44:03 had antibodies that reacted with B*44:02 (35). This patient was sensitized by the donor's C*07:04, which carries the 156DA eplet shared with B*44:02 and a group of HLA-B alleles, including B*08:01, B*37:01, B*41:01, B*42:01, B*44:02, B*45:01, and B*82:01. All of them reacted with patient's serum. In another case, the 151ARE eplet

on a C*02:02 mismatch had induced antibodies that reacted with all 151ARE-carrying HLA-B alleles (36). These findings demonstrate that sensitization induced by an epitope on a HLA-C mismatch can cause other class I antigens to become unacceptable mismatches because they share that epitope, although the patient may have never been exposed to such antigens.

As another example, patients sensitized by a DR2 mismatch often have antibodies reacting with DR1 (37). Such antibodies are induced by DR51, which is in strong linkage disequilibrium with DR2. They are specific for the 96EV eplet shared between DR51 and DR1. Conversely, sensitization by a DR1 mismatch can lead to 96EV-specific antibodies that react also with DR51 but not with DR2. These findings point towards the importance of DRB3/4/5 eplets in DRB-specific antibody responses of kidney transplant recipients (38).

Clinical relevance of epitope-based mismatch acceptability for patients with pre-transplant HLA antibodies

The highly sensitized patient represents an enigma for kidney transplantation—not only is it difficult to find a suitably matched donor, but subsequent kidney transplants are often less successful. The analysis of serum reactivity for HLA antibodies has two clinical goals. Most commonly used is the identification of antibody-defined HLA antigens that should be considered unacceptable. This system is designed to identify donors who must be excluded but it does not necessarily mean that all other HLA antigens would be compatible for a patient. The other goal is to determine HLA antigens that are acceptable mismatches. This strategy represents a direct approach of finding a compatible donor for a sensitized patient (39).

HLAMatchmaker has been applied to the analysis of serum antibody reactivity of sensitized patients and the identification of potential donors with acceptable epitope mismatches (40-43). This approach shortens the waiting time for a suitable kidney donor and leads to excellent graft survivals comparable to those seen with non-sensitized recipients. Eurotransplant has incorporated

HLAMatchmaker in the Acceptable Mismatch program to identify donors for highly sensitized patients (42, 44) and a similar program is now being investigated for implementation in the European Union (Frans Claas, personal communication).

Other investigators have also reported the usefulness of HLAMatchmaker in the selection of suitable transplant donors (43, 45-47) and the management of refractory thrombocytopenic patients in need of platelet transfusions (48-50).

Effect of HLA-ABC epitope load on the class I antibody response

HLAMatchmaker can be used as a quantitative tool to determine the degree of a mismatch, i.e. the number of mismatched eplets. Each HLA antigen mismatch has an epitope load that is primarily determined by the recipient's HLA type representing a repertoire of self-epitopes to which no antibodies can be made. As illustrated in Table 1, a mismatched antigen might be structurally compatible for some phenotypes but not for others.

There is now considerable evidence that anti-class I antibody responses induced by a transplant or during pregnancy correlate with the number of non-self epitopes on mismatched antigens (36, 43, 51-55) (72). Table 2 summarizes these findings.

Minimizing the epitope loads of antigen mismatches is also beneficial in the HLA-based platelet transfusion support of alloimmunized thrombocytopenic patients (48, 49). An HLA epitope based matching protocol may lead to a more effective platelet transfusion management (56). This protocol includes high-resolution HLA-ABC typing of patients and donors, serum screening to identify acceptable mismatches, and the identification of suitable donors in a donor database that incorporates HLAMatchmaker as a search engine.

Effect of HLA-DRDQ epitope load on the class II antibody response

Conventional class II matching criteria consider only HLA-DR antigen, but this approach is an insufficient reflection of histocompatibility because

Table 1. Examples of how the eplet load of an antigen mismatch depends on the HLA phenotype of the recipient

Case	Recipient HLA phenotype				Donor	#Eplets	Mismatched Eplets on Molecular Surface
	A*02:01	B*07:02	B*13:02	C*06:02			
1	A*01:01	B*07:02	B*13:02	C*06:02	A*26:01	11	62RNR,62RR,62RRN,62RTN,63NN,66NV,145RT,149TAH,151AHE,163RW,245AS
2	A*01:01	B*07:02	B*08:01	C*07:01	A*26:01	13	62RNR,62RR,62RRN,63NN,145RT,149TAH,151AHE,156WA,184A,193AV,194V,207S,245AS
3	A*02:01	B*15:01	B*45:01	C*02:02	A*26:01	12	62RRN,63NN,65RNA,66NV,66NAH,69ATN,71STN,73TDA,76ANT,77NGT,163R,163RW
4	A*01:01	B*07:02	B*57:01	C*04:01	A*26:01	16	62RNR,62RR,62RRN,62RTN,63NN,145RT,149TAH,150AH,151AHE,156WA,163RW,184A,193AV,207S,245AS,253Q
5	A23:01	B*41:01	B*44:03	C*08:02	A*26:01	1	73TDA
6	A*01:01	B*44:02	B*39:01	C*01:02	A*26:01	5	145RT,149TAH,151AHE,163RW,245AS
7	A*24:03	B*55:01	B*37:01	C*06:02	A*26:01	4	73TDA,76ANT,77NGT,79GT
8	A*01:01	B*35:01	B*49:01	C*04:01	A*26:01	0	none
1	A*01:01	B*07:02	B*13:02	C*06:02	B*51:01	8	44RT,66IF,76ENI,80I,163L,163LE,163LW,170RH
2	A*01:01	B*07:02	B*08:01	C*07:01	B*51:01	16	44RT,71TTN,73TY,76EN,76ENR,76ENI,80I,81ALR,82LR,131S,163L,163LE,163LW,170RH,193PV,194V
3	A*02:01	B*15:01	B*45:01	C*02:02	B*51:01	11	44RT,62RNQ,66IF,71TTN,76EN,76ENR,76ENI,80I,81ALR,82LR,170RH
4	A*01:01	B*07:02	B*57:01	C*04:01	B*51:01	5	44RT,66IF,69TNT,71TTN,170RH
5	A23:01	B*41:01	B*44:03	C*08:02	B*51:01	8	44RT,62RNQ,66IF,76ENI,80I,163LW,170RH
6	A*01:01	B*44:02	B*39:01	C*01:02	B*51:01	6	44RT,66IF,76ENI,80I,163LW,170RH
7	A*24:03	B*55:01	B*37:01	C*06:02	B*51:01	6	66IF,71TTN,163L,163LE,163LW,170RH
8	A*01:01	B*35:01	B*49:01	C*04:01	B*51:01	1	170RH
1	A*01:01	B*07:02	B*13:02	C*06:02	C*05:01	7	69RT,71ATN,73TDV,73TVN,138K,177KT,275G
2	A*01:01	B*07:02	B*08:01	C*07:01	C*05:01	15	62RK,63EK,65QKR,66K,69RT,71ATN,73TVN,80K,138K,177KT,184H,193PV,194V,267PE,275G
3	A*02:01	B*15:01	B*45:01	C*02:02	C*05:01	4	138K,156RA,177KT,275G
4	A*01:01	B*07:02	B*57:01	C*04:01	C*05:01	1	73TVN
5	A23:01	B*41:01	B*44:03	C*08:02	C*05:01	3	71ATN,73TVN,80K
6	A*01:01	B*44:02	B*39:01	C*01:02	C*05:01	5	71ATN,73TVN,138K,177KT,275G
7	A*24:03	B*55:01	B*37:01	C*06:02	C*05:01	6	71ATN,73TVN,138K,156RA,177KT,275G
8	A*01:01	B*35:01	B*49:01	C*04:01	C*05:01	10	1C,69RT,70QT,71ATN,73TDV,73TV,73TVN,138K,177KT,275G

Table 2. Correlations between epitope loads and HLA class I antibody responses.

Year	Investigators	Reported Observation	Reference
2002	Lobashevsky et al. (Alabama)	Numbers of epitope (triplet) mismatches predict flow cytometry crossmatch results with sera from highly sensitized renal patients ($p < 0.00009$).	(51)
2004	Dankers et al. (Leiden)	Correlation between the number of mismatched epitopes (triplets) and the incidence of humoral sensitization induced by a kidney transplant ($r^2 = 0.99$, $p < 0.0001$) or developed during pregnancy ($r^2 = 0.95$, $p < 0.0001$)	(52)
2006	Goodman et al. (Cambridge, UK)	Correlation between the number of mismatched epitopes (triplets) and the presence of HLA antibodies detected in Luminex assays with single class I alleles	(43)
2008	Kosmoliaptis et al. (Cambridge, UK)	Analysis of recipient HLA type and mismatched HLA antigens using the HLAMatchmaker algorithm allows prediction of immunogenic donor HLA types.	(54)
2009	Kosmoliaptis et al. (Cambridge, UK)	Close correlation between increasing number of amino acid polymorphisms and the presence and magnitude of the HLA antibody response ($p < 0.0001$)	(72)
2011	Duquesnoy et al. (Pittsburgh)	More HLA-C antibody responses by transplant patients who have been exposed to greater HLA-C eplet loads ($p < 0.001$)	(36)
2013	Schaub et al. (Basel, Switzerland)	Number of mismatched HLA-ABC eplets strongly correlates with the rate of child-specific class I sensitization ($p < 0.001$).	(55)

antibodies against other class II mismatches, including DP and DQ, have been shown to diminish transplant success. Each DR antigen should be viewed as a package of DR+DQ+DP antigens and the overall class II epitope load depends on the patient's DR, DQ, DP type.

As an example, let us assume that DR11,DR16 corresponds to DRB1*11:01, DRB3*02:01, DQB1*03:01, DQA1*05:01/ DRB1*16:01, DRB5*02:02, DQB1*05:02, DQA1*01:02 genotype and that the serological DR antigens correspond to the common DR-DQ haplotypes shown as reported in (57). The DR12 and DR15 haplotype mismatches have the lowest eplet loads, namely 6 and 9, whereas the DR7 and DR9 haplotypes have 28 and 27 mismatched eplets, respectively. For a D15, DR18 patient, the DR1 and DR8 haplotypes have 9 and 4 mismatched eplets and the DR4 and DR7 haplotypes have 42 and 41 mismatched eplets. These examples illustrate that high-resolution typing can provide detailed class II matching information at the eplet level that some DR-DQ mismatches have low epitope loads.

Donor-specific, DRB1-reactive antibodies are less common than antibodies against other class II epitopes and this correlates with lower numbers of mismatched DRB1 eplets (38). In contrast, donor-specific DRB3/4/5 mismatches induce more antibody responses and they have higher

numbers of incompatible eplets. Antibodies against HLA-DQ are also more common and this is not surprising since DQB and DQ antigens have more mismatched eplets on than DRB1 antigens (38). Recently, Nickerson's group reported that locus-specific epitope mismatches were more numerous in patients who developed to donor-specific antibodies against HLA-DR (21.4 versus 13.2, $p < 0.02$) or HLA-DQ (27.5 versus 17.3, $p < 0.001$) (58). They concluded that HLA-DR and DQ epitope matching outperforms traditional antigen-based matching and has the potential to minimize the risk of de novo Class II donor-specific antibody development.

HLA epitope load and organ transplant outcome

In the 1990s, several reports by Terasaki's group pointed out the influence of mismatched epitope numbers on kidney transplant survival (6, 59, 60). A 2003 analysis of the United Network for Organ Sharing and Eurotransplant kidney transplant databases showed that HLA-A,B mismatched kidneys with low epitope (triplet) loads have the same graft survival rates as the zero HLA-A,B antigen mismatches (61). Triplet matching has also provided useful prognostic information about kidney transplantation in African-Americans (62). On the other hand, a study of the Collaborative

Transplant Database showed no significant association between triplet matching and kidney graft survival (63), but another look at these data showed clearly similar five-year graft survivals for the zero-antigen mismatches and groups with zero or few triplet mismatches (64). Taylor's group reported that single HLA-A or -B mismatched grafts with no or one amino acid mismatch had better survival than grafts with two or more amino acid mismatches (89.3% versus 81.8% at 5 years, $p = 0.03$) (65). The number of mismatched amino acids was an independent predictor of transplant survival ($p = 0.02$). Laux and co-workers reported that mismatching for DPB at the epitope level had an adverse effect on kidney transplant survival (65).

Böhringer and co-workers showed that class I epitope (triplet)-based matching is associated with a better prognosis of penetrating keratoplasty and reduces the time on the waiting list for most patients awaiting a corneal transplant (66, 67). They developed an HLAMatchmaker-based tolerogenicity formulation determined from the numbers of mismatched HLA-A,B and HLA-DR eplets (68). It was based on optimal thresholds of favorable tolerogenic factors calculated as $(\# \text{ mismatched DR eplets})^2$ minus $(\# \text{ mismatched A,B eplets})^2$ as less than 220 and fewer than 10 mismatched HLA-A,B eplets in one cohort of 586 normal-risk patients. In a second cohort of 975 consecutive low-risk and high-risk patients, these threshold estimates correlated with better rejection-free survival rates. The authors suggest that an operational tolerance might be inducible by balanced matching of HLA-class I and II eplets (68).

A National Marrow Donor Program study of 744 unrelated hematopoietic cell transplantation cases with one HLA-ABC mismatch and 1690 fully HLA-ABC, DRDQ allele matched cases showed that class I epitope (triplet) matching had a very modest effect on engraftment and reduced graft versus host disease but did not improve patient survival (69). This finding is not surprising because stem cell transplants are primarily affected by cellular immune mechanisms, whereas HLAMatchmaker addresses only antibody-defined

epitopes. Moreover, epitope (triplet) matching does not predict in vitro alloreactive cytotoxic T-cell responses (70).

Immunogenicity of HLA epitopes

How immunogenic are individual HLA epitopes? It is well known that sensitized patients develop specific antibodies to restricted numbers of mismatched epitopes. A practical approach is to collect information about the frequencies of epitope-specific antibody responses in context with the exposure rate to epitope mismatches (71). A 14th International HLA Workshop study on 44 patients with rejected kidney transplants who had undergone allograft nephrectomy showed high frequencies of cytotoxic antibodies to several eplets that are well exposed on the molecular surface whereas eplets with low immunogenicity are in less accessible positions (71).

Two paradigms have been proposed for HLA epitope immunogenicity. One deals with the physiochemical properties of amino acid polymorphisms of HLA. Kosmoliaptsis and co-workers reported that antibody responses to HLA mismatches depend not only on the number of foreign amino acids but also on differences in physiochemical disparity related to hydrophobicity and electrostatic charge of the polymorphic amino acids (54, 72). Their molecular and physiochemical HLA modeling studies showed different surface electrostatic motifs for the serological Bw4 and Bw6 epitopes and offer new insights into HLA epitope immunogenicity (73).

Our experience with antibody-verified eplet pairs has shown that the immunizing allele has one eplet that is nonself whereas as the other is always a self eplet shared with the antibody producer (19, 20). This suggests an autoreactive component of the alloantibody response to an HLA mismatch and a recent report has expanded this view to the so-called nonself-self paradigm of eplet immunogenicity (74). This paradigm is based on the hypothesis that B-cells carry low-affinity immunoglobulin receptors for self-HLA epitopes. Their interactions with self HLA will not lead to B-cell activation or antibody production. In contrast,

exposure to HLA mismatches often induces strong alloantibody responses. This means that the activation of a self-HLA specific B-cell by a non-self eplet requires that the remainder of the structural epitope on the immunizing antigen be identical or very similar to the corresponding self HLA epitope of the antibody producer. Three recent studies have provided experimental support of this hypothesis (74, 75).

These findings raise the possibility that the antibody response to a mismatched eplet requires that the remainder of the structural epitope on the immunizing HLA antigen must have a very similar amino acid composition as the corresponding self epitope of the antibody producer. Any antigen with significant residue differences with patient's self structural epitopes might not be able to activate HLA-specific B-cells and an HLA antibody response. Future research may show that the nonself/self paradigm of eplet immunogenicity and the physiochemical properties of epitopes would be clinically useful regarding predicting antibody responses to HLA mismatches.

CONCLUSION

This chapter summarizes the evidence for the clinical usefulness of epitope-based HLA matching in the transplant setting. Several transplant programs apply this concept in finding acceptable mismatches for highly sensitized patients and this has resulted in better transplant outcomes and the identification of more suitable donors. Prevention of HLA sensitization represents a significant challenge for the non-sensitized transplant candidate. There are new opportunities because we can now determine epitope loads of mismatched antigen and begin to recognize the highly immunogenic epitopes. Epitope loads permit risk assessments for antibody-mediated rejection and in the current clinical setting this information will be useful in the monitoring and management of patients who have received a transplant. Epitope-based matching should eventually lead to new strategies for HLA mismatch permissibility to reduce alloimmunization and increase transplant survival.

SUMMARY

Human leukocyte antigen (HLA) mismatches are important risk factors for antibody-mediated rejection and transplant failure. With the realization that HLA antibodies recognize epitopes rather than antigens, it has become apparent that donor-recipient compatibility should be assessed at the epitope level. Recent developments have increased our understanding of the structural basis of HLA antigenicity (i.e. the reactivity with specific antibody) and immunogenicity (i.e. the ability to induce an antibody response). HLAMatchmaker is a computer algorithm that considers each HLA antigen as a series of

small configurations of polymorphic residues, referred to as eplets, as essential components of HLA epitopes. This chapter addresses the relevance of determining epitope specificities of HLA antibodies in the identification of acceptable mismatches for sensitized patients considered for transplantation. Permissible mismatching for non-sensitized patients aimed to prevent or reduce HLA antibody responses could consider epitope loads of mismatched antigens and the recently developed concepts about the physiochemical basis of immunogenic epitopes and the nonself-self paradigm of eplet immunogenicity.

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