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Editorial

HLA epitope based matching for transplantation

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SUMMARY

As important risk factors for transplant rejection and failure, HLA antibodies are now recognized as being specific for epitopes which can be defined structurally with amino acid differences between HLA alleles. Donor–recipient compatibility should therefore be assessed at the epitope rather than the antigen level. 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. It includes epitopes on antigens encoded by all HLA-A, B, C, DR, DQ and DP loci as well as MICA. HLA epitopes have two characteristics namely antigenicity, i.e. the reactivity with antibody and immunogenicity, i.e. the ability of eliciting an antibody response. This article addresses the relevance of determining epitope-specificities of HLA antibodies, the effect of epitope structure on technique-dependent antibody reactivity and 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 nonself-self paradigm of epitope immunogenicity.

1. Introduction

HLA antibodies are strongly associated with transplant rejection and decreased graft survival. They are produced following exposure to mismatched HLA antigens and this can happen after transplantation, during pregnancy and following blood transfusion. HLA antibodies are also the principal cause of alloimmunization-induced refractoriness of thrombocytopenic patients who require platelet transfusions from compatible donors. Such antibodies were originally described as antigen-specific (e.g. anti-A1, anti-B7 and anti-DR1) or specific for serologically cross-reacting antigen groups such as the A2-CREG and the B7-CREG. It is now known that HLA antigens carry multiple epitopes which can be defined by structural modeling of HLA molecules and comparisons of amino acid sequence differences between alleles. HLAMatchmaker is a computer algorithm which is based on HLA compatibility at the epitope level. Each HLA antigen is considered as a string of amino acid configurations as key elements of epitopes that can elicit specific alloantibodies. The original version used triplets, i.e. linear sequences of three residues [1]. The so-called eplet version is based as described below on stereochemical modeling of protein antigen–antibody complexes and the contributions of critical amino acid residues that dominate in antigen–antibody binding [2]. HLAMatchmaker programs consider Class I [2], Class II [3], and MICA compatibility and antibody analysis [4]. The www.HLAMatchmaker.net website is an information resource and has various epitope-based analysis programs that can be downloaded free of charge. A 16th International Histocompatibility and Immunogenetics Workshop project has led to the website-based Registry of Antibody-Defined HLA Epitopes (http://www.epregistry.com.br) [5]. 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 [6–8].

The www.HLAMatchmaker.net website has Microsoft Excel programs to analyze serum reactivity patterns for epitope-specific antibodies. The Teresina group in Brazil has developed the user-friendly EpHLA software programs designed for clinical laboratories to analyze antibody reactivity patterns and assess epitope mismatch acceptability [9,10]. Antibody analysis programs by commercial vendors of Luminex HLA antibody kits consider also epitopes.

Three recent reviews describe the concept of HLAMatchmaker and its usefulness in HLA epitope matching for organ transplantation [11–13].

2. 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 is viewed as a collection of epitopes that can be determined according to general concepts on how the paratope of antibody binds with a protein epitope. The paratope has three heavy chain and three light chain Complementarity Determining Region (CDR-H1, -H2, -H3, -L1, -L2 and -L3) loops that contact a protein epitope. The interphase consists of multiple amino acid residues distributed over a surface area of 700–900 square Ångstroms [14–17]. There are about 15–25 contact residues in what has been referred to

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as a structural epitope and a centrally located so-called functional epitope consisting of a few residues binds with CDR-H3 which plays a dominant role in determining antibody specificity [18–21]. HLAMatchmaker considers eplets equivalent to functional epitopes and that they are centrally located within the context of a structural epitope contacted by other CDRs of antibody. Based on the 700–900 square Ångstrom dimensions of protein epitopes one can estimate that contacting residues must reside on the HLA molecular surface within a radius of about 15 Ångstroms of an eplet [2]. Analogous to functional epitope dimensions, 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. Each eplet is annotated by a position number in the amino acid sequence and polymorphic residues shown with the standard single letter code. 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).

The HLA Epitope Registry (http://www.epregistry.com.br) has 270 HLA-ABC eplets on alleles used in Luminex assays for antibody testing, and 219 are in antibody-accessible positions on the molecular surface. There are 51 eplets defined solely by residue polymorphisms located below the molecular surface but it remains to be determined how many of them can elicit specific antibodies. The repertoires of class II antigens include 147 DRB1/3/4/5, 57 DQB1, 24 DQA1, 43 DPB1, 15 DPA1 and 67 MICA eplets.

HLA epitopes have two distinct characteristics namely antigenicity, i.e. their reactivity with antibody, and immunogenicity, i.e. their ability of inducing an antibody response. In both cases we must consider that each HLA epitope is determined by an eplet in context with the corresponding structural epitope. These concepts are important not only for a basic understanding of antibody-defined HLA epitopes but also clinically relevant regarding HLA antibody analysis and assessments of mismatch acceptability and permissibility for transplant recipients.

3. HLA epitope antigenicity

HLA antibodies are specific for eplets in context with other amino acid configurations within 15 Ångstroms, the presumed locations of residues in corresponding structural epitopes that contact CDRs. Information about HLA types of antibody producer and immunizer is important for the determination of epitope specificity of antibodies. Sensitization to a mismatched eplet can induce specific antibodies with different reactivity patterns with allele panels as measured in antigen binding assays on a Luminex platform. This sensitive test gives mean fluorescence intensity (MFI) values as indicators of antibody reactivity. Monospecific antibodies may have the following reactivity patterns:

First, an antibody has high MFI values with all eplet-carrying HLA alleles including the immunizing allele whereas the other alleles including self-alleles have extremely low MFI values and are thus nonreactive. Such antibody is specific for an epitope solely defined by an eplet. It is possible and even seems likely that other residues within 15 Ångstroms are involved as contact sites but they cannot be identified because in this instance, they would be the same on all eplet-carrying alleles.

Second, an antibody has a high MFI values with certain eplet-carrying alleles including the immunizer but negative MFI values with other eplet-carrying alleles and the other alleles in the panel. Our studies with class I specific antibodies have shown that the reactive eplet-carrying alleles must have a second polymorphic residue configuration which serves as a critical contact site for antibody [22,23]. Such antibody is specific for an epitope defined as an eplet pair. Interestingly, these pairs largely involve self configurations present in the HLA type of the antibody producer. Eplet pair defined epitopes have also been reported on HLA-DQ dimers whereby one eplet resides on the DQA chain and the other one is on the DQB chain [24,25].

Third, an antibody shows different MFI values with eplet-carrying alleles ranging from high (including the immunizing allele) to low and very low. They could reflect distinct residue configurations on lowly reacting alleles that interfere with antibody binding but not necessarily cause negative MFI values [26]. Differences between MFI values with eplet-carrying alleles may also be seen with so-called peptide dependent HLA antibodies [27–30].

It should be noted that many structural epitopes have residue variations that do not significantly alter reactivity with epitope-specific antibodies [22]. In other words, antibody specificity is generally directed to functional epitopes (eplets) in combination of certain dominant residues in corresponding structural epitopes.

In conclusion, eplets are key elements of HLA epitopes but additional residues can be expected to affect reactivity with specific antibodies.

4. Effect of epitope structure on technique-dependent HLA antibody reactivity

Residue differences within eplet-defined structural epitopes may also explain technique-dependent variations in antibody reactivity. Recent studies have compared the reactivity patterns of human HLA class I epitope-specific cytotoxic antibodies in Ig-binding, C1q-binding Luminex assays and by complement-dependent lymphocytotoxicity testing with large panels [31]. Some antibodies react similarly with epitope-carrying alleles in all three assays but others showed different reactivity patterns primarily Ig-binding positive + C1q-binding and CDC negative or Ig-binding and C1q-binding positive + CDC negative. These technique-dependent reactivity patterns are associated with polymorphic residue variations in structural epitopes of corresponding eplets.

These findings have been viewed in the context of the role of multiple CDR contact sites in antigen–antibody complex formation that results in the release of free energy necessary to stabilize binding and to induce conformational changes in the antibody molecule to expose the C1q binding site, the first step of complement activation. Moreover, the amount of free energy should be sufficient to induce a conformational change of C1q thereby initiating the first stages of the classical complement cascade leading to lymphocytotoxicity. The complement-fixing properties of HLA antibodies require not only specific recognition of eplets but also depend on interactions of other CDRs with critical amino acid configurations within the structural epitope. Eplet-carrying alleles that lack such configurations may only bind with antibody. This concept is important to our understanding whether or not complement-fixing donor-specific HLA antibodies can initiate antibody-mediated rejection.

5. HLA epitope specificity analysis in the clinical setting

The application of HLAMatchmaker to the analysis of serum reactivity may increase our understanding of otherwise unexplained sensitization patterns induced by a given mismatch. Sensitization to certain HLA-C mismatches can lead to antibodies reacting with epitopes shared with HLA-B antigens [32,33]. For instance, anti-156DA antibodies induced by C*07:04 react also with 156DA-carrying B*08:01 and B*44:02. These findings demonstrate that exposure to an epitope on a HLA-C mismatch may 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. HLAMatchmaker can also explain unexpected reactivity patterns of class II antibodies. For instance, patients sensitized by a DR2 mismatch have often antibodies reacting with DR1 and this not due to a cross-reactivity between DR1 and DR2 [34]. 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 antibodies that react also with DR51 but not with DR2. These findings

illustrate the importance of DRB3/4/5 epitopes in DRB-specific antibody responses of kidney transplant recipients [35].

HLA-DQ and HLA-DP heterodimers have distinct eplet repertoires and certain DQ and DP eplets react often with class II antibodies [35,36]. DQ antibodies can also recognize pairs of eplets shared between DQA and DQB chains [25,37].

HLAMatchmaker can be used to identify epitopes recognized by anti-MICA antibodies and the determination of MICA mismatch acceptability [4]. Molecular viewing of the MICA structure and the determination of amino acid sequence differences between MICA alleles has yielded a repertoire of 38 potentially immunogenic MICA eplets. Early MICA panels could not distinguish between antibodies specific for eplets in two supereplets [4] also referred by Stastny as groups G1 and G2 [38]. Larger MICA panels are now available that can identify the eplets in these group [8].

6. HLA mismatch acceptability for sensitized patients

HLAMatchmaker is a useful tool in the analysis of serum antibody reactivity of sensitized patients and the identification of potential donors with acceptable epitope mismatches [39–42]. 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 [41,43] and a similar program (Eurostam) 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 [42,44–46] and the management of refractory thrombocytopenic patients in need of platelet transfusions [47–49].

7. HLA immunogenicity and epitope load

HLAMatchmaker can be used as a quantitative tool to determine the degree of a mismatch, i.e. the number of mismatched eplets. A given 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 alloantibodies can be made. For some patients, a mismatched antigen might be structurally compatible whereas for other patients it has multiple mismatched epitopes [11].

The incidence of the anti-class I antibody response induced by a transplant or during pregnancy correlates with the number of non-self triplets or eplets on mismatched antigens [42,50–53]. Anti-HLA-C antibody responses by patients with rejected kidney transplants also correlate with eplet loads of HLA-C mismatches [33].

Conventional class II matching criteria consider only HLA-DR antigens but this approach is an insufficient reflection of class II compatibility because antibodies against other class II mismatches including DP and DQ have been shown to diminish transplant success. Each DR antigen must be viewed as a package of DR + DQ + DP antigens and the overall class II epitope load depends on the patient's DR, DQ, and DP type [11]. As an example, let as assume that DR11,DR16 corresponds to the 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 DRB1-DRB3/4/5-DQB-DQA haplotypes. The DR12 and DR15 haplotypes have the lowest eplet loads namely 6 and 9, whereas DR7 and DR9 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 and that some DR–DQ mismatches have low epitope loads.

Epitope loads of class II mismatches affect specific antibody responses. Donor-specific, DRB1-reactive antibodies are less often detectable than antibodies against other class II epitopes [35]. DRB3/4/5 mismatches induce more antibody responses than DRB1 mismatches and this corresponds to higher numbers of incompatible eplets. Anti-DQ antibodies are rather common and this correlates with more mismatched eplets on DQB and DQA than on DRB1 [35].

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 vs. 13.2, $p < 0.02$) or HLA-DQ (27.5 vs. 17.3, $p < 0.001$) [54]. 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.

In summary, information about epitope loads of HLA mismatches seems clinically useful in the management of transplant patients. Epitope loads can be interpreted as risk factors for antibody-mediated rejection and for each transplant recipient this information could be provided to the transplant physicians. Eventually, epitope load information might be incorporated in new strategies to identify donors with HLA permissible mismatches to reduce alloimmunization and increase transplant success.

An HLA epitope based matching protocol may lead to a more effective platelet transfusion management [55]. This protocol includes high-resolution HLA-A,B,C 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.

8. HLA epitope load and transplant outcome

A 2003 analysis of the UNOS 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 [56]. Triplet matching has also provided useful prognostic information about kidney transplantation in African–Americans [57]. On the other hand, a study of the Collaborative Transplant Database showed no significant association between triplet matching and kidney graft survival [58] but another look at these data showed clearly similar five-year graft survivals for the groups with zero or few triplet mismatches and the zero-antigen mismatches and [59]. 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 whereas the number of mismatched amino acids was an independent predictor of transplant survival [60]. Laux and et al. at the Collaborative Transplant Database reported that mismatching for DPB at the epitope level had an adverse effect on kidney transplant survival [61].

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 [62]. 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 [63].

9. 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 frequencies of epitope-specific antibody responses in context with the exposure rate to epitope mismatches [64]. 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 [64].

Two paradigms have been proposed for HLA epitope immunogenicity. One deals with the physiochemical properties of amino acid polymorphisms of HLA and the other is based on the nonself-self residue composition of structural HLA epitopes. Kosmoliaptsis et al. 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. [52,65]. 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 [66].

10. Nonself-self paradigm of HLA epitope immunogenicity

Our experience with antibody-verified epitopes defined by eplet pairs have shown that the immunizing allele has one eplet that is nonself whereas as the other part is always a self configuration also present in the HLA type of the antibody producer [22,23]. This suggests that the alloantibody response to an HLA mismatch must have an autoreactive component but how can this be explained? The answer must consider the nonself-self theory of the immune response originally forwarded by Burnet [67–69] and extensively discussed by many investigators [70–77].

During B-cell development, rearrangements of V_H and V_L genes produce diversity in the antigen-binding sites of immunoglobulins [78,79]. These processes lead to the expression of immunoglobulin receptors on developing B-cells which go through several stages to become mature B-cells. These receptors can recognize epitopes on autologous proteins [80]. B-cells with high-avidity receptors disappear after positive and negative selection [81–83] and receptor editing [84,85] and the remaining B-cells carry only low-avidity receptors so that their interactions with autologous proteins will not induce their activation. An antibody response cannot be triggered by every foreign entity, but it requires a “criterion of continuity” of antigenic patterns (whether endogenous or exogenous) which discriminate nonself from self [76]. In other words, the immune system does not react to self molecules, but will respond to certain modifications within self molecules.

The nonself-self paradigm of HLA epitope immunogenicity is based on the hypothesis that each person had a repertoire of B-cells with low-avidity immunoglobulin receptors for epitopes on their own HLA molecules [86]. Their interactions with self HLA eplets will not lead to B-cell activation or antibody production. In contrast, exposure to HLA mismatches with nonself eplets may induce alloantibody responses. This means that the remainder of the immunizing structural epitope must 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 [86,87]. We studied sixteen eplet-specific antibodies and each case, at least one allele of the antibody producer had no or very few differences with the immunizing allele in antibody-accessible positions defined by a 15 Å radius of the mismatched eplet, the presumed dimension of a structural epitope.

The nonself-self paradigm of HLA epitope immunogenicity may also explain unexpected reactivity patterns of epitope-specific antibodies. Two transplant candidates had antibodies induced during pregnancy against an epitope defined by the 145R + 82LR pair on the immunizing B*44:02 [87]. Patient 1 had 145R on her A*30:01, A*66:01 and B*14:02 alleles and patient 2 had 145R on her A*11:01, B*07:02, C*06:02 and C*07:02 alleles. These patients typed as B*13:02 which has 82LR. In other words, HLAMatchmaker considers both eplets as intralocus and/or interlocus matches which should not induce antibodies in these patients. On the other hand, B*13:02 allele has 145L rather than 145R. This would mean that the 145R + 82LR pair is different from 145L + 82LR and as an immunogenic epitope it had activated B-cells with receptors for self 145L + 82LR. This conclusion is consistent with the results of comparative sequence analysis which showed that B*13:02 and the immunizing B*44:02 had exactly the same residues within a 15 Ångstrom radius of 145L + 82LR [87]. There are similar cases whereby the antibody response to an epitope can only be explained with the nonself-self paradigm of HLA epitope immunogenicity (unpublished data).

These findings support the concept that HLA antibodies originate from B-cells with self-HLA immunoglobulin receptors that recognize mismatched eplets as nonself entities on immunizing antigens. The humoral alloresponse to an HLA mismatch is not well understood. It is well known that sensitized patients develop specific antibodies to a restricted number of mismatched epitopes [88,89]. The nonself-self paradigm of HLA epitope immunogenicity may explain this phenomenon. It is possible that the antibody response to an eplet requires that its structural epitope on the immunizing HLA antigen must be structurally very similar to the corresponding self epitope of the antibody producer.

Conversely, any antigen with a significant structural epitope difference with patient's self epitopes might not be able to activate B-cells to produce HLA antibodies. The nonself-self concept of HLA immunogenicity may become clinically useful regarding predicting antibody responses to HLA mismatches but it needs of course, experimental verification in the clinical setting.

11. Conclusions

HLA matching at the epitope level offers an attractive approach to identify acceptable mismatches for sensitized patients. Prevention of HLA sensitization represents a significant challenge for the non-sensitized transplant candidate. Two causes, namely blood transfusions and the transplant itself could be approached as being potentially controllable. Perfect HLA matching is somewhat impractical because it can be done for small numbers of patients. On the other hand, information about epitope loads and an understanding of epitope immunogenicity may be useful in assessing risks for antibody-mediated rejection after transplantation and the clinical management of transplant recipients. Such permissible mismatching may lead to new strategies to identify suitable donors with minimal risks for allosensitization.

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