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16th IHIW: A Website for Antibody-Defined HLA Epitope Registry

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Summary

The concept that HLA antibodies are specific for epitopes rather than HLA antigens is important not only for the determination of mismatch acceptability for sensitized patients but also for a better understanding of the antibody response to an HLA mismatch. Numerous publications describe epitope-specific antibodies, but there is no standardized information about the repertoire of clinically relevant HLA epitopes. Under auspices of the 16th IHIW, we have developed a website-based registry of antibody-verified HLA epitopes. Epitope notations are based on HLA molecular modelling of amino acid residues in polymorphic sequence positions. Informative epitope-specific antibodies had been induced by a transplant, transfusion or pregnancy and were monoclonal antibodies or eluates of sera absorbed with single HLA alleles. Antibody reactivity was determined in binding assays with single-allele panels. Antibody producer/immunizer HLA types enhanced the characterization of specific epitopes. The Registry also includes epitopes described in original research publications. Based on the extent of antibody reactivity information, we assigned epitope status as confirmed (well documented) or provisional (more data are needed). At present, the Registry has 69 HLA-ABC, 53 DRB1/3/4/5, 17 DQ, 8 DP and 22 MICA antibody-verified epitopes and will be updated on a quarterly basis. Laboratories worldwide continue to submit data about previously unreported antibody-specific epitopes. For each epitope, the website shows its amino acid composition and HLA alleles that share the epitope. Links show antibody reactivity patterns, sensitization information and references. Other links show molecular modelling of corresponding structural epitopes and polymorphic residue information for epitope-carrying alleles. The website will also have a link to epitope frequency information in different populations. Search functions will list mismatched epitopes on mismatched alleles for selected HLA types. The HLA Epitope Registry will become a valuable resource for researchers interested in HLA compatibility at the epitope level and investigating antibody responses to HLA mismatches.

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Introduction

While it is generally accepted that HLA antibodies represent significant risk factors for transplant rejection and graft failure, it has become apparent that such antibodies are specific for epitopes rather than HLA antigens. A distinction between HLA epitopes and antigens is important not only for the determination of mismatch acceptability for sensitized patients but also for a better understanding of the humoral immune response to a HLA mismatch. Detailed information about HLA molecular structure and amino acid sequences has made it possible to determine the structural basis of HLA epitopes. However, true existence of epitopes can only be proven with specific antibodies. There is already a considerable literature about antibody-reactive HLA epitopes, but no comprehensive database is available or a standard notation for such epitopes. Moreover, the overall repertoire of clinically relevant HLA epitopes seems incomplete, and there is a need for identifying new epitopes with specific antibodies.

Under auspices of the 16th International HLA and Immunogenetics Workshop, we have initiated a collaborative project that has led to the establishment of a website-based registry of antibody-defined HLA epitopes. This project does not address so-called 'cellular HLA epitopes' defined by alloreactive T cells. The development of a database of clinically relevant HLA epitopes presented a considerable challenge.

First of all, what annotation system should be used? One possibility is a numbering system for epitopes for their respective HLA genes as has been used by Terasaki's group (El-Awar *et al.*, 2007, 2009). Another approach is to annotate epitopes with amino acid polymorphisms in antibody-accessible sequence positions on the HLA molecular surface as described in the HLAMatchmaker algorithm (Duquesnoy, 2006, 2008).

Second, what methods should be used to identify antibody-defined epitopes? It seems that antibody binding assays such as Luminex with single-allele panels should be used as a minimum, but what about other methods such as ELISA and Complement-dependent lymphocytotoxicity ?Obviously, the HLA panels used for antibody testing must be informative enough for epitope specificity analysis, but how do we address the problem of inconsistencies between antibody reactivity patterns for the different methods?

Finally, what are the criteria for 'monospecificity' of antibodies for the identification of epitopes? Human anti-HLA monoclonals are ideal sources especially if HLA information is available for antibody producer and immunizer; however, their availability is limited. Mouse anti-HLA monoclonals are somewhat useful, although many react with xenoepitopes that are different from clinically relevant (allo) epitopes. Sera from sensitized patients appear to be valuable sources of epitope-specific antibodies, but absorption–elution studies with informative single alleles are generally necessary to obtain monospecific antibodies.

Altogether, a classification of antibody-defined epitopes depends on the antibody reactivity pattern with an informative HLA panel, but this is not a simple matter. In contrast to the definition of HLA alleles that are solely based on amino acid or nucleotide differences in any sequence position, antibody-defined HLA epitopes have different criteria. HLA epitopes might be structurally equivalent to distinct amino acid configurations in antibody-accessible positions. Moreover, epitopes are determined by their binding with specific antibody, and these epitope–paratope complexes result from multiple physiochemical binding forces that involve the complementarity determining regions (CDRs) of antibody heavy and light chains. Although many antibody-defined HLA epitopes correspond to simple well-established amino acid configurations, one must expect many others have a considerably more complex composition. We have kept these issues in mind during the development of a website-based registry of HLA epitopes.

Description of the HLA epitope registry website

A dedicated website (http://epregistry.ufpi.br) has been developed in the Department of Informatics and Statistics, Federal University of Piauí (Teresina, Brazil). This group has also developed a software program for epitope analysis of HLA antibodies (Sousa *et al.*, 2011). The website provides access to five separate epitope databases: Class I ABC, DRB1/3/4/5, DQB + DQA, DPB + DPA and MICA. There are also instructions for laboratories how to submit data about new antibody-defined epitopes and an (optional) registration for people who wish to have periodic updates about the Registry.

Each layout of the five epitope databases has the following displays:

(1) *Epitope names* have distinct sequence position numbers and polymorphic residue descriptions with standard single-letter amino acid codes. We have tried to keep these notations as simple as possible, and each database displays a complete as possible set of configurations as potential epitopes recognized by antibody. For instance, the ABC repertoire has more than 200 potential epitopes present on class I alleles in Luminex panels. Each epitope has its own row, and epitopes are sorted according to their sequence positions. Possible variants such as an epitope pair or other molecular configurations affecting reactivity with antibody are shown in separate lines under the associated epitopes. More rows will be used to describe additional variants as they are identified.

(2) *Polymorphic residue descriptions* show the residue names and sequence positions that cluster together on each epitope. For instance, the HLA-A2, B57 and B58 alleles in the Luminex panel share a distinct epitope 62GE, which can be described by a 62G (glycine), 63E (glutamic acid) and 65R (arginine). Some antigens have more than one unique epitope in different sequence positions. For instance, the A1 and A36 alleles in the Luminex panels share three unique epitopes: 44KM (described by 44Q, 44K, 45M and 46E), 149A + 150V + 151H and 158V. The epitope notation is $44KM_3$, whereby the subscript indicates the possibility of three distinct epitopes that cannot be distinguished by the Luminex panels currently used for antibody testing.

The well-known epitope 82LR (described by 79R, 82L and 83R) is present on all Bw4-associated HLA-B antigens and HLA-A23, A24, A25 and A32. Although many antibodies have been reported to be monospecific for 82LR, others recognize related epitopes present on subgroups of 82LR-carrying antigens that share additional unique residue configurations. For instance, certain antibodies react with all 82LR-carrying HLA-B antigens but not with the 82LR-carrying HLA-A23, A24, A25 and A32; these HLA-B antigens share a glutamine residue (Q) in position 43, whereas the HLA-A antigens have a phenylalanine or 43P. The corresponding epitope is therefore a pair called 82LR + 43Q. Other antibodies have been shown to react with all 82LR-carrying antigens except A25 and B13, and the corresponding epitope requires the presence of a distinct three-residue configuration around position 145;

this epitope can be annotated as 82LR + 145RAA. In the reactivity analysis of antibody-defined epitopes, the HLA typing information about the antibody producer and the immunizer permits a determination of which parts of pairs are self-configurations; they have the preface 's', for example, 82LR + s145RAA. These examples illustrate how the annotation system of the Registry can describe structurally complex epitopes.

The epitopes of other HLA loci have similarly formatted names and residue descriptions. Separate class II epitope databases are used for DRB1/3/4/5, DQB + DQA and DPB + DPA. For instance, DRB1*01, DRB5*01 and DRB5*02 share the 96EV epitope described by the cluster 96E, 98K and 180V in discontinuous sequence locations. HLA-DQ and HLA-DP molecules are α,β heterodimers, and the Registry has epitope annotations for all of them. For instance, DQB1*02 and *03:02 (DQ8) share the 56PA epitope and DQA1*02, *04 and *06 have the 75IL epitope. DQ epitopes can also be defined by two configurations, one on the β-chain and the other on the α-chain of the heterodimer (Tambur *et al.*, 2010). One such epitope is defined by 56PPD on DQB1 and 74SNL on DQA1 and is named 56PPD-a74SNL. It should be noted that DQ epitopes are assigned only to DQB1-DQA1 molecules and not to DQB2-DQA2, which are not expressed on the cell membrane.

HLA-DP epitopes are listed according to chains and sequence locations. For instance, the DPB1*03, *14 and *28 alleles share the 65LK epitope, and DPA1*02 and *04 share the 50RA epitope. Although cross-reacting epitopes shared between different DR, DQ and DP antigens are rare, there are some notable exceptions. As an example, antibodies to 57DE on DRB1*11 alleles often cross-react with 55DE on DPB1*02, *03, *04:02, *06, *09, *10 *14, *16, *17, *18, *20 and *28. The names of such epitopes will have 'x' suffixes, for example, 57DEx and 55DEx, and cross-reactivity patterns are further detailed in reactivity pattern descriptions for antibody-verified epitopes.

There is now considerable evidence that MICA antibodies are associated with transplant rejection, and the Registry has a database of MICA epitopes. For instance, antibodies have been identified as specific for 24T shared by MICA alleles A*001, A*012 and A*018.

(3) *Epitope frequencies*: They have been calculated from the HLA phenotype registry of the National Marrow Donor Program. We are also planning to establish a link to Derek Middleton's website on HLA allele frequencies (http://www.allelefrequencies.net), to generate epitope frequencies in different population groups.

(4) *Reactivity patterns of antibody-defined epitopes*: As described previously, each database comprises a list of potential epitopes that can be predicted to serve as recognition sites for specific antibodies. This Registry is intended to document epitopes that have been verified experimentally with informative specific antibodies. Depending on the completeness of antibody reactivity information, there are two categories of antibody-defined epitopes: 'confirmed' or 'provisional'. Analogous to original serological assignments of HLA antigens used during the early international workshops, the provisional status will be upgraded if sufficient additional verification becomes available.

For each antibody-defined epitope, a link box on the webpage provides information about antibody reactivity pattern as well as antibody source and, if available, information about the immunizing event including HLA types of antibody producer and immunizer. Recorded antibody reactivity patterns are primarily based on direct binding assays (Luminex) preferably with single-allele panels. There is a considerable amount of published information about antibody-defined epitopes that has been recorded on the website. These links cite investigators providing antibody reactivity information as well as local names of epitopes and specific literature references. Figure 1 shows an example of a link about the reactivity pattern of an antibody-defined epitope.

An important aspect is the participation by HLA professionals who have identified antibodies specific for new and not so well-described epitopes. From the website, one can download forms to submit epitope-specific antibody reactivity information, which will be reviewed before recording in the Registry.

(5) *Information about corresponding 'structural' epitopes*: A distinction between epitope antigenicity (the ability to react with antibody) and immunogenicity (the ability to induce an antibody response) is an important consideration (Duquesnoy, 2008, 2011). Although many HLA epitopes are believed to consist of configurations of small numbers of amino acid residues, we must consider the well-known fact that antibodies have three heavy chain CDR and three light chain CDR loops that make contact with so-called 'structural epitopes' comprising 15–25 residues distributed over surface areas of 700–900 $Å^2$. Centrally located, so-called 'functional epitopes' comprise small configurations of amino acid residues, and they play a dominant role in determining epitope specificity. HLA-Matchmaker-defined eplets can be considered equivalents to functional epitopes. Each eplet must have a corresponding structural epitope with additional surface residues (estimated to be within a 15-Å radius) that contact the CDR loops of antibody.

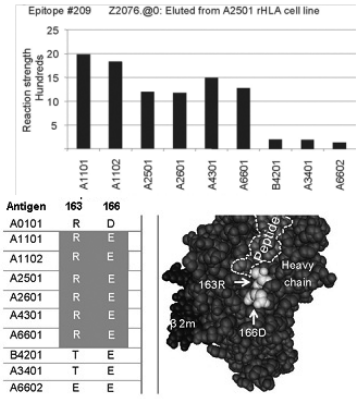
One can expect two reactivity patterns. First, an antibody reacts with all eplet-carrying alleles with structural epitopes consisting of contact residues that are monomorphic or have permissive polymorphisms. Second, an antibody reacts with a subgroup of eplet-carrying alleles, because these alleles have structural epitopes that have contact residues which are critical for binding with antibody. Eplet-carrying alleles that lack such critical contact sites will not bind significantly with antibody. Studies on human monoclonal antibodies have provided experimental support of this concept (Duquesnoy *et al.*, 2005; Marrari *et al.*, 2010;

Epitope: 163RE (confirmed)

Luminex single allele reactivity: **Human monoclonal antibody VP5G3 (IgM) from Arend Mulder (Leiden):** Sensitization due to pregnancy. Antibody producer types as HLA-A24, 32; B18, B60; Cw10. Immunizing haplotype is HLA-A26; B55; Cw1.
Data supplied by Marilyn Marrari (Pittsburgh):

PANEL		OL MFI	GP MFI
Positive control		nt	nt
Negative control		9	5
163 RE+	A*11:01	11150	8163
163 RE+	A*11:02	12567	4246
163 RE+	A*25:01	13029	7194
Immunizing allele: 163 RE+	A*26:01	12521	9514
163 RE+	A*43:01	7510	1165
163 RE+	A*66:01	12498	11044
Self Alleles		21 ± 11	9 ± 4
Other 163RE-negative alleles		84 ± 278	9 ± 3

Data from El-Awar et al, *Human Immunology* 68: 170-180, 2007: Serum eluate after absorption with A*25:01 cells



Antigen	163	166
A0101	R	D
A1101	R	E
A1102	R	E
A2501	R	E
A2601	R	E
A4301	R	E
A6601	R	E
B4201	T	E
A3401	T	E
A6602	E	E

Other antibody assays: **Data from Mulder et al, *Tissue Antigens* 52: 393-396, 1998:** VP5G3 reacts with HLA-A11, A25, A26, A66 in CDC assays; A43 not tested.
Data from Fernandez-Vina et al, in Immunobiology of the Human MHC vol. I, John A. Hansen (Ed), pp 890-931, 2006 VP5G3 (13-WS 0061) positive with HLA-A*11:01, *11:02, *11:03, *24:10, *25:01, *25:02, *26:01, *26:02, *26:03, *26:08, *26:15, *66:01 in CDC assays in 13th IHWS. No HLA-A43-positive cells were tested.

Comments: Equivalent to TerEp #209.

Figure 1. Example of the reactivity pattern of an antibody-verified HLA epitope.

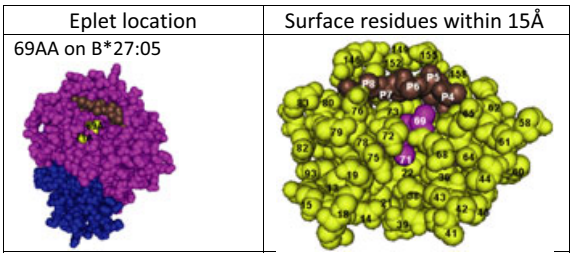
Tambur *et al.*, 2010). Such antibodies are specific for HLA epitopes defined by pairs of eplets no more than 15 Å apart; for these pairs, one eplet is generally non-self, and the other is a self-eplet shared between antibody producer and immunizer.

Critical contact sites can be identified by comparing the structural epitope polymorphisms of the immunizing allele and the alleles in Luminex panel.

Figure 2 is an example of a structural epitope link. It shows a Cn3D model of the location of 69AA epitope on a B*27:05 molecule, and within 15 Å, the surface residues as potential contact sites with antibody. The table shows polymorphic residue differences between 69AA-carrying alleles in the Luminex panel and how this information can be used to explain antibody reactivity with a subset of 69AA-carrying alleles. Suppose, B*07:02 was the immunizing allele and the antibody reacts with all 69AA-carrying alleles except B*15:16 and B*57:01; the latter have 46A, but the reactive alleles have 46E, apparently a critical contact site. This antibody would be specific for an epitope defined by the 69AA + 46E pair. The critical contact site would be a self-configuration if the HLA type of the antibody producer has 46E. Other scenarios could be that the 69AA on B*07:02 would generate antibodies that react with all 69AA-carrying alleles except B*27:03 (59Y seems critical) or except B*15:16, B*57:01 and B*58:01 (62R, 65Q and 66I seem critical) or except B*73:01 (76E seems critical) or except B*15:16, B27:03, B*27:05, B*57:01 and B*58:01 (80N, 82R and 83G seem critical), etc. Thus, information about polymorphic residue differences appears useful in characterizing the molecular configuration of more complex antibody-defined epitopes.

The structural epitope of 69AA has also contact residues on the HLA-bound peptide (Figure 2). Arend Mulder and colleagues have first demonstrated that certain human monoclonal HLA antibodies are peptide dependent (Mulder *et al.*, 2005). One can expect that the alleles of the immunizer and the Luminex panel have different peptide repertoires and that the binding of some antibodies will require contact with certain critical residues on the peptide. Differences in expression levels of such residues will affect the binding of epitope-carrying alleles with peptide-dependent antibodies.

Altogether, the structural epitope links will be useful in the interpretation of antibody reactivity patterns especially for the identification of new epitopes. Moreover, they may help to explain differences in antibody reactivity patterns between Ig and C1q binding Luminex assays as well as complement-dependent lymphocytotoxicity.



Polymorphic surface residues within 15Å									
Position	46	59	62	65	66	76	80	82	83
B*07:02	E	Y	R	Q	I	E	N	R	G
B*15:16	A	Y	R	R	N	E	I	L	R
B*27:03	E	H	R	Q	I	E	T	L	G
B*27:05	E	Y	R	Q	I	E	T	L	G
B*27:08	E	Y	R	Q	I	E	N	R	G
B*42:01	E	Y	R	Q	I	E	N	R	G
B*54:01	E	Y	R	Q	I	E	N	R	G
B*55:01	E	Y	R	Q	I	E	N	R	G
B*56:01	E	Y	R	Q	I	E	N	R	G
B*57:01	A	Y	G	R	N	E	I	L	R
B*58:01	E	Y	G	R	N	E	I	L	R
B*73:01	E	Y	R	Q	I	V	N	R	G
B*81:01	E	Y	R	Q	I	E	N	R	G
B*82:02	E	Y	R	Q	I	E	N	R	G

Figure 2. Example of a structural epitope and a description of residue polymorphisms.

(6) *Epitope-carrying alleles in Luminex panels*. This includes all potential and antibody-defined epitopes.

(7) *Listing of all alleles with antibody-defined epitopes*. This includes all four-digit alleles with expressed proteins as recorded in recent HLA nomenclature reports.

Search functions. Each epitope database webpage has search options to identify selected repertoires of antibody-defined epitopes. Searching by a given allele will open a new webpage with all antibody-defined epitopes on that allele. Entering the HLA type of recipient will open a new webpage showing all mismatched antibody-defined epitopes for that recipient. This search can be performed separately for each database. Entering information for both search mechanisms will generate a webpage displaying an allele's mismatched antibody-defined epitopes for this recipient. Each webpage is readily available for printing or to generate PFD files.

Discussion

The HLA Epitope Registry is a work in progress. At present, the repertoire of antibody-defined epitopes is very incomplete. We plan to update the Registry with new antibody-identified epitopes and additional information about epitope status quarterly, and we invite all HLA professionals to submit informative data about HLA-reactive antibody reactivity patterns.

The Registry does not address the clinical relevance of HLA epitopes in transplantation. As a start, we might conclude that any HLA epitope that elicits an antibody response would be potentially relevant. This depends on the immunoglobulin types and functional ability of antibodies to elicit inflammatory responses leading to rejection. In this regard, the reactivity of complement-fixing antibodies might be influenced by the structure of the HLA antigen that carries the epitope specifically recognized.

Epitopes are named and described with sequence positions and polymorphic residues, but their exact molecular configurations remain imprecise. The experimental verification of HLA epitopes is performed with physiochemical assays such as Luminex binding of specific antibodies, but often enough, it is difficult to distinguish between positive, weakly positive and negative reactions. These variations in binding strength appear often due to residue differences between epitope-carrying alleles, but again little is known about their clinical relevance.

The application of the 'structural epitope' concept seems useful in the characterizing more complex epitopes, but the residue polymorphisms of current Luminex panels may not always be informative enough. HLA 'epitopology' studies are needed to understand the molecular basis of HLA antigen–antibody complexes and the reactivity of various alleles including those with residue substitutions induced by site mutagenesis.

Nevertheless, the HLA Epitope Registry will become a valuable resource for researchers interested in HLA compatibility at the epitope level and investigating antibody responses to HLA mismatches.

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