

Human Monoclonal Antibody Reactivity With Human Leukocyte Antigen Class I Epitopes Defined by Pairs of Mismatched Eplets and Self-Eplets

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Aim. Humoral sensitization affects transplant outcome, and it is now apparent that human leukocyte antigen (HLA) antibodies are specific for epitopes rather than antigens. Such epitopes can be structurally defined by HLAMatchmaker, an algorithm that considers eplets as critical elements of epitopes recognized by alloantibodies. This study addressed the question how mismatched HLA antigens induce specific antibodies in context with eplet differences with the antibody producer.

Methods. HLA class I–specific human monoclonal antibodies derived from women sensitized during pregnancy were tested in Luminex assays with single allele panels. Their epitope specificity was determined from reactivity patterns and eplet differences between immunizing antigen and the antibody producer.

Results. This study focuses on the reactivity patterns of 10 monoclonal antibodies specific for epitopes defined by a mismatched eplet paired with a self-eplet shared between immunizing HLA antigens and HLA antigens of the antibody producer. The eplets in these pairs are between 7 and 16 Å apart, a sufficient distance for contact by two separate complementarity-determining regions of antibody.

Conclusions. These findings demonstrate that immunizing antigens have mismatched eplets that can form antibody-reactive epitopes with self-configurations on the molecular surface. They seem to suggest that HLA antibodies can be produced by autoreactive B cells that have undergone receptor editing to accommodate the recognition of nonself-eplets, the driving force of the humoral alloresponse. This concept enhances our understanding of structural epitope immunogenicity and the interpretation of antibody reactivity patterns with HLA panels.

Keywords: HLA epitope, Monoclonal antibody, Eplet, HLAMatchmaker, Epitope structure, Class I HLA antigen.

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Although it is generally accepted that human leukocyte antigen (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 an HLA mismatch. There are two strategies to define such epitopes.

One is based on the analysis of antibody reactivity patterns with HLA panels and the identification of amino acid configurations shared between reactive alleles. Terasaki's group in particular has conducted extensive studies of antibodies tested with single HLA alleles on a Luminex platform (1, 2).

HLAMatchmaker is a theoretical algorithm developed from stereochemical modeling of protein antigen-antibody complexes (3–5) whereby each HLA antigen is viewed as a collection of polymorphic amino acid configurations within a 3-Å radius in antibody-accessible positions; these so-called eplets are considered key elements of epitopes that can elicit specific alloantibodies.

We have recently reported a comparative analysis how amino acid-defined epitopes described by Terasaki's group correspond to HLAMatchmaker defined eplets (6, 7). Approximately one half of 103 Terasaki's class I epitopes (TerEps) are equivalent to eplets. Approximately 10% of them correspond to eplets with certain dominant residues, whereas the other polymorphic residues of the eplet can be substituted without any effect on epitope specificity.

Approximately one third of class I TerEps correspond to pairs of eplets. Although no HLA typing information of the antibody producer and the immunizer was available, we postulated that the eplets in such pairs could be both nonself or,

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alternatively, one is nonself and the other is self. The latter is analogous to our findings with human monoclonal antibodies (mAbs) specific for epitopes defined by combinations of a nonself-eplets and a self-eplet separated far enough for contact by two different complementarity-determining regions (CDRs) of antibody (3, 8). Nonself-eplets would function as specificity recognition sites for antibody, and the self-eplets serve as critical contact sites with another CDR of antibody. Accordingly, nonself-eplet carrying alleles that lack the critical contact site do not react with antibody.

This report provides further evidence that HLA alloantibodies can recognize epitopes defined by mismatched eplets paired with self-eplets shared between the immunizer and the antibody producer.

RESULTS

This study focuses on human mAbs specific for epitopes defined by eplet pairs. We have previously reported three such epitopes on the immunizing A3 antigen; in each case, one eplet was nonself and the other was a self-eplet also present on the antibody producer's own HLA antigens (8). A recent HLAMatchmaker analysis of class I epitopes described by Terasaki's group (1) has shown that approximately 30% of them correspond with eplet pairs, but because no HLA typing information was available about the antibody producer and immunizer, we could not determine what eplets in these pairs were self or nonself (6).

This report describes 10 mAbs specific for epitopes that correspond to eplet pairs (Table 1). From the HLA typing information of the antibody producer, we have determined that in all pairs one eplet is nonself and the other eplet is self. We describe here two examples in more detail.

The 82LR eplet corresponds to the well-known Bw4 epitope shared by a group of HLA-B antigens and A23, A24, A25, and A32. For instance, mAb MUS4H4 came from a woman who typed as HLA-A2, 66; B39, 41 and developed antibodies reacting with the paternal A24 of the child. This mAb reacted specifically with all 82LR carrying HLA-A and

HLA-B antigens. The 82LR eplet is equivalent to epitope 24 reported by Terasaki's group (5).

On the other hand, some 82LR-specific antibodies show different reactivity patterns. The B37-induced VDK8F7 came from a woman who typed as A3, 31; B35, - and reacted with the paternal B37 of the child and specifically all 82LR-carrying antigens except A25 and B13 (Fig. 1). All reactive 82LR-carrying antigens have the same eplet in sequence position 145, namely 145RAA. In contrast, the negatively reacting A25 and B13 have 145RTA and 145LAA, respectively. This shows that 145RAA is a major component of the epitope recognized by VDK8F7. This eplet is present on all HLA-A and HLA-B antigens of the antibody producer and is therefore considered self. Thus, VDK8F7 recognizes an epitope described by 82LR+s145RAA. As illustrated in Figure 1, these eplets are approximately 14 Å apart and could serve as contact sites for two different CDRs of antibody.

HDG4B1 originated from an antibody producer who types as A2, 24; B7, 60 who was sensitized to an A32 mismatch. This mAb reacts with A25, A32, B57, B58, and B63; all of them share 65RNA with the immunizer (Fig. 2). However, the 65RNA-carrying A1, A3, A11, A26, A28, A29, A30, A31, A33, A34, A36, A43, A66, A74, and A80 as well as all 65RNA-negative antigens do not react. This suggests that the epitope recognized by HDG4B1 is related to 65RNA but must include an additional configuration uniquely shared between A25, B57, B58, and B63 and the immunizing A32. Indeed, these antigens share 82LR, whereas all negatively 65RNA-carrying antigens have 82RG. In this case, 82LR is a self eplet present on A24 of the antibody producer and is important for the reactivity of HDG4B1 with 65RNA-carrying antigens. Thus, HDG4B1 is specific for 65RNA+s82LR; these eplets are approximately 11 Å apart, a sufficient distance for contact with two separate CDRs of antibody (Fig. 2).

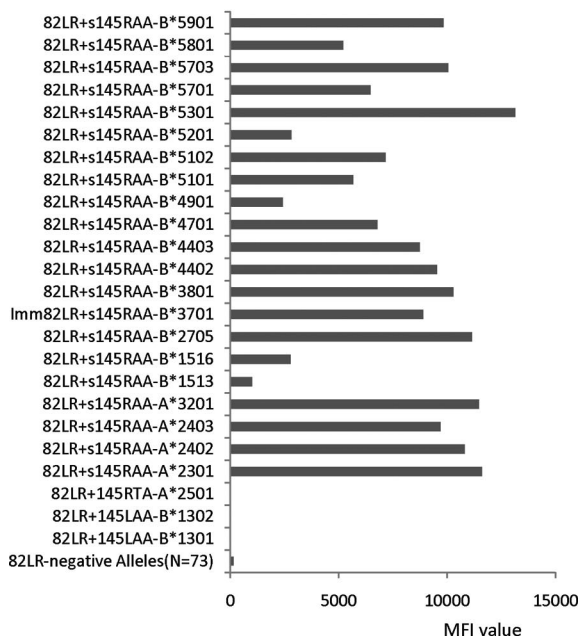
Table 1 summarizes the reactivity of 10 mAbs specific for pairs of nonself and self-eplets: four are IgM-type and six are IgG-type, and all of them reacted with groups of antigens. Two mAbs MUL4C8 and BRO11F6 came from dif-

TABLE 1. Human mAbs with reactivity patterns corresponding to eplet pairs

mAb name	Isotype	HLA type antibody producer	Immunizing antigen	Eplet specificity	Distance	Reactive antigens	Self-eplet on
VDK8C7	IgM	A3, 31; B35,-; Cw4,-	B37	82LR+s145RAA	14 Å	All Bw4 except A25 and B13	All alleles
HDG41B1	IgG	A2, 24; B7, 60; Cw7, w10	A32	65RNA+s82LR	11 Å	A25/A32/B17/B63	A24
MUL4C8	IgG	A2, 25; B18, 51; Cw12, 15	A11	144KR+s151H	7 Å	A1/A3/A11/A24/A36	A2, A25
BRO11F6	IgG	A26, 68; B38, 44; Cw7	??	144KR+s151H	7 Å	A1/A3/A11/A24/A36	A26, A68
MUL9E11	IgG	A2, 25; B18, 51; Cw12, 15	B55	69AAT+s76E	8 Å	B7/B27/B42/B54/B55/B56/ B57/B58/B63/B67/B81/B82	A2, B18, B51, Cw12, Cw15
MUL9F4	IgM	A2, 25; B18, 51; Cw12, 15	B55	65QIA+s76ES	7 Å	B7/B*2708/B42/B54/B55/ B56/B67/B81/B82	A25, B18
OK2H12	IgM	A2, 28; B7, 27; Cw2, w7	A3	62QE+s56G	11 Å	A1/A3/A11/A32/A36/A74	All alleles
OK4F10	IgM	A2, 28; B7, 27; Cw2, w7	A3	142MI+s79GT	13 Å	A1/A3/A11/A26/A29/A30/ A31/A33/A34/A36/A43/A66/ A74/A80	A2, A28
WK1D12	IgG	A1,-; B8,-; Cw7,-	B27	163EW+s73TE	16 Å	B7/B13/B27/B40/B47/B48/B81	B8
HDG11G12	IgG	A2, 24; B7, 60; Cw7, w10	B35	163LW+s62RQI	8 Å	B15/B35/B49/B50/B51/ B52/B53/B56/B78	B7, B60

mAbs, monoclonal antibodies; HLA, human leukocyte antigen.

VDK8F7 reactivity



Epitope: 82LR+s145RAA

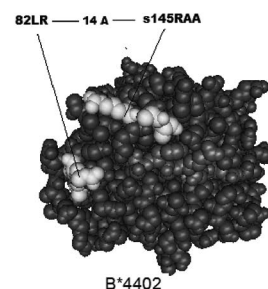
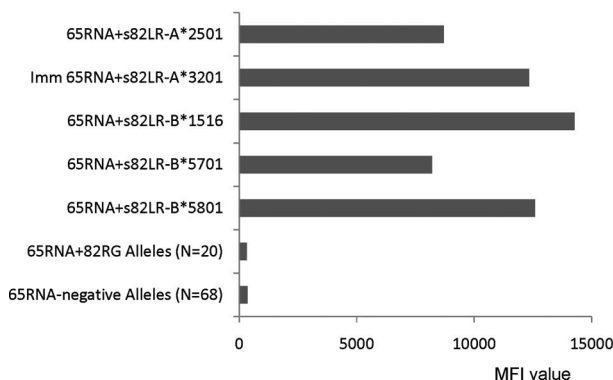


FIGURE 1. Reactivity of B37-induced VDK8F7 monoclonal antibody with single allele Luminex panel. The molecular structure of the 82LR+s145RAA epitope is depicted on a B*4402 crystal model (PDB 1M6O). Median fluorescence intensity, MFI.

HDG41B1 reactivity



Epitope: 65RNA + s82LR

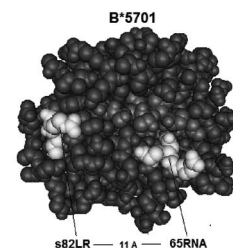


FIGURE 2. Reactivity of A32-induced HDG41B1 monoclonal antibody with single allele Luminex panel. The molecular structure of the 65RNA+s82LR epitope is depicted on a B*5701 crystal model (PDB 2RFXX). Median fluorescence intensity, MFI.

ferent antibody producers but have identical reactivity with all 144KR+s151H-carrying antigens. The B55-induced MUL9F4 and MUL9E11 are specific for two distinct eplets paired with different self-configurations in sequence position 76, namely the 76ES eplet and a glutamic acid residue, respectively. The A3-induced OK2H12 and OK4F10 have been tested previously in ELISA and lymphocytotoxicity assays (8); their Luminex patterns showed the same specificity, namely 62QE+s56G and 142MI+s79GT, respectively. The immunizing antigen of WK1D12 was B27, and this mAb is specific for 163EW paired with s73TE found on B8 of the antibody producer. The B35-induced HDG11G12 is specific for another eplet in position 163, namely 163LW paired with s62RQI present on the B8 and B60 of the antibody producer. Molecular modeling has shown that in these pairs, the mismatched eplet and the self-configuration were approximately 7 to 16 Å apart.

DISCUSSION

Although many antibody-reactive HLA epitopes appear identical to HLAMatchmaker-defined eplet mismatches (5), this study demonstrates that other epitopes consist of pairs of eplets whereby one is mismatched and the other one is a self-configuration shared between the immunizing antigen and one or more HLA antigens of the antibody producer. This self-configuration could be an eplet or a single residue. Alleles with the same mismatched eplet but paired with a different configuration do not react or at best, are very weakly reactive.

In these pairs, the distance between the mismatched eplets and self-configurations ranges from 7 to 16 Å. Considering the overall epitope-paratope surface of 700 to 900 Å² (9–11), this distance is consistent with the concept that the eplets in these pairs make contact with different CDRs of antibody. The mismatched eplet interacts with the CDR that

plays a primary role in the specificity of antibody, whereas the self-eplet (or configuration) serves as a critical contact site with another CDR of antibody. The latter is important for the formation of a stable antigen-antibody complex.

These findings provide a new insight of eplet immunogenicity. Some antibodies react with all antigens carrying a mismatched eplet originally presented by the immunizing antigen. Other antibodies recognize an eplet but only in context with a self-configuration in a polymorphic sequence location. Therefore, we can conclude that a given mismatched eplet can induce specific antibodies with different reactivity patterns. One pattern pertains to all antigens that carry a given eplet specifically recognized. Another pattern involves eplet-bearing antigens that share a given self-configuration of amino acids, which is critical for the binding with antibody. Thus, there seems to be an autoreactive component of the alloantibody response to some HLA mismatches.

These findings might be viewed in context with current concepts of antibody structure and B-cell diversity (12). Antibody specificity is determined by the variable regions of immunoglobulin heavy (V_H) and light (V_L) chains, each one has three hypervariable loops representing the binding surface with epitope. This surface is usually 700 to 900 Å² and is comparable to that of an HLA molecule seen from the top. During B-cell development, rearrangements of various V_H and V_L genes produce diversity in the antigen-binding sites of immunoglobulins. These processes take place in the absence of antigen but require nonlymphoid stromal cells in the bone marrow. They lead to the expression of immunoglobulin receptors on developing B cells, which go through several stages: from pro-B cells to pre-B cells to immature B cells and finally mature B cells. The latter migrate to the secondary lymphoid organs where they undergo further differentiation. The membrane-bound immunoglobulin functions as a specific receptor for antigen, and on encountering antigen, the B cell is stimulated to proliferate and differentiate into plasma cells that secrete specific antibody.

Developing B cells acquire a vast repertoire of immunoglobulin receptors with different antigenic specificities including those for self-epitopes on normal tissue proteins. The latter are considered predominant selective forces for the primary repertoire (13, 14), but developing B cells are also programmed to generate negative signals that cause inactivation and apoptosis leading to clonal deletion of many autoreactive cells. However, the immunoglobulin receptors on autoreactive immature B cells can also be modified by successive V_L gene rearrangements referred to as receptor editing (15–17). Accordingly, immature B cells exchange the self-reactive L chain for one that is not self-reactive, whereas the successful V_H gene rearrangements are mostly maintained during the progression to mature B cells (18, 19). Thus, B-cell receptors will keep some of their self-reactivity, but their engagement with self-protein is insufficient for B-cell activation. On the other hand, such B-cell receptors may recognize altered forms of proteins with amino acid substitutions, and this would ultimately lead to antibody production. This condition may apply to the alloantibody response.

It is possible that HLA antibody-producing cells originally had B-cell receptors with CDR loops that can interact with self-HLA constituents, whereas other CDR loops are the result of receptor editing to accommodate the recognition of

non-self-eplets on mismatched HLA antigens. This concept explains the antibody specificity against HLA epitopes defined by a pair of nonself and self-eplets. It may also increase our understanding of epitope immunogenicity and the antibody response patterns to HLA mismatches, which seem enhanced by self-recognition. Antibody reactivity to eplet pairs raises also questions about determining mismatch acceptability for sensitized patients. If the patient has antibodies against a mismatched eplet paired with self-configuration, can we consider an eplet-carrying allele as an acceptable mismatch if it lacks this self-configuration?

In the clinical setting of transplantation, it has become apparent that HLA epitopes rather than antigens are important for matching and analyzing antibody specificity. This notion is particularly relevant to the determination of mismatch acceptability for sensitized patients, especially if a potential donor types for alleles not included in the antigen panel used for antibody screening. Although many antibody-reactive epitopes have been characterized structurally, it remains to be seen which ones are clinically relevant and how many more epitopes will be identified. Considering the importance of epitope matching in transplantation, there is a need to establish a database for clinically relevant epitopes. A 16th International Histocompatibility Workshop project is underway to address this issue.

MATERIALS AND METHODS

Human mAbs

This study addresses the reactivity patterns of human mAbs generated as previously described (20–22). All of them are derived from Caucasoid women (Dutch) who became sensitized during pregnancy by paternal HLA antigens of their children. Complement-dependent cytotoxicity testing with HLA-typed lymphocyte panels was used to select supernatants of cloned hybridomas generated from Epstein-Barr virus-transformed B cells. HLA types of antibody producers and immunizers were determined by standard serologic and molecular methods.

Determination of HLA Antibody Reactivity

This was done with microbead Luminex assays using single HLA class I allele kits from two commercial vendors (One Lambda Inc., Canoga Park, CA; Gen-Probe, LifeCodes Corporation, Stamford, CT) according to manufacturer's instructions. In brief, an aliquot of a mixture of Luminex microspheres, each coated with a single antigen, was incubated with a small volume of test serum sample and washed to remove unbound antibody. Anti-human immunoglobulin (IgG or IgM) antibody conjugated to phycoerythrin was added; after incubation, the bead mixture was diluted for analysis with a Luminex 100 instrument (Luminex, Austin, TX), and the reactivity was determined with the manufacturer's software. Median fluorescence intensity (MFI) values were recorded for each allele and the positive and negative control beads. All mAbs showed extremely low MFI values (mostly <100) with the self-alleles of the antibody producer. A given allele was considered mAb reactive if the MFI values were consistently high in comparison with the immunizing antigen and the positive controls.

Determination of Epitope Specificity

We have conducted an HLA Matchmaker analysis to determine which eplets were shared by mAb reactive alleles. This is a Microsoft Excel program that has worksheets to enter the HLA types of the antibody producer, the immunizer, and the allele panel as well as the MFI values that can be readily copied from CSV files of the manufacturer's Luminex computer programs. The antibody analysis program described on the website <http://www.HLAMatchmaker.net> has been modified to incorpo-

rate the concept that pairs of eplets that are about or less than 15 Å apart can represent certain epitopes.

An understanding of antibody reactivity requires information about the immunogenetic basis of the sensitizing event leading to the formation of specific antibody. HLAMatchmaker can determine on the immunizing antigen the repertoire of eplets that are mismatched for the antibody producer. This analysis is based on the obvious assumption that each mAb is specific for a single epitope presented by the immunizing antigen. The first step is to record into the program those alleles that give negative reactions with antibody. Such alleles can be expected to have epitopes that are not recognized by antibodies, and these epitopes are automatically removed from further consideration as potential specificity sites on antibody-reactive alleles. In the reactive alleles, we then determine which eplets are shared with the immunizing allele.

Structural Modeling of Epitopes

We determined the locations of epitopes on the HLA molecular surface using an extensive collection of crystallographic structures downloaded from the Entrez Molecular Modeling Database on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/Structure>). This was done with the Cn3D structure and sequence alignment software program, which has a “select by distance” (in Å) command that determines how far eplets are apart (23).

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