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Correlations between Terasaki's HLA class I epitopes and HLAMatchmaker-defined eplets on HLA-A, -B and -C antigens

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Abstract

Although the determination of human leukocyte antigen (HLA) antibody specificity has traditionally been directed toward HLA antigens, there is now increasing attention to structurally defined HLA epitopes. An understanding of the HLA epitope repertoire is important to acceptable mismatching for sensitized patients and to a new epitope-based matching algorithm aimed to reduce antibody-mediated rejection. There are two strategies to determine the HLA epitope repertoire. Terasaki's group has used an empirical method to analyze the reactivity of single allele Luminex panels with mouse monoclonal antibodies (mAbs) and absorbed/eluted alloantibodies with a computer program based on shared residues in the amino acid sequences of reactive alleles. HLAMatchmaker is a theoretical algorithm that predicts HLA epitopes on the HLA molecular surface from stereochemical modeling of epitope–paratope interfaces of antigen–antibody complexes. Our epitope repertoire is based on so-called 'eplets' representing 3-Å patches of at least one polymorphic residue on the molecular surface. A comparative analysis has shown that 81/103 Terasaki's HLA class I epitopes are equivalent to individual eplets ($n = 50$) or pairs of eplets ($n = 31$) separated far enough to serve as potential contact sites for two complementarity-determining regions of antibody. An additional 12 Terasaki's epitopes (TerEps) correspond to eplets with permissible residue combinations that do not seem to affect epitope specificity. We could not identify corresponding eplets for the remaining 10 TerEps, including 8 that might be considered xeno-epitopes defined by mouse mAbs. Conversely, HLAMatchmaker has 38 additional eplets in well-exposed surface positions that do not have equivalent TerEps, and for many of them, we have found specific antibodies. These findings strengthen the concept that eplets are essential basic units of HLA epitopes and that they provide a better understanding of HLA immunogenicity (i.e. ability to induce an antibody response) and antigenicity (i.e. reactivity with specific antibody).

Introduction

There is now overwhelming evidence that antihuman leukocyte antigen (HLA) antibodies cause transplant rejection and decrease organ transplant survival. These antibodies are usually detected in sera from sensitized patients, and their specificity has traditionally been defined toward HLA antigens, many of which can be assigned to serologically cross-reacting groups. The elucidation of the three-dimensional molecular structure and detailed information of amino acid sequence differences have led to the concept that HLA antigens have multiple epitopes that are determined by amino acid residues in polymorphic positions. Numerous reports have addressed the amino acid composition of HLA epitopes recognized, especially by mouse monoclonal antibodies (mAbs).

During recent years, the development of sensitive antibody detection assays has provided a new direction regarding the clinical significance of anti-HLA antibodies in

transplantation. An important component is the determination of the epitope repertoire on the HLA molecular surface because this information may lead to a new epitope-based matching algorithm aimed to control antibody-mediated rejection.

There are two strategies to determine the HLA epitope repertoire. One uses an empirical approach. Terasaki's group has performed extensive analyses of antibody reactivity patterns in antigen-binding tests such as the sensitive Luminex assay with recombinant HLA single antigen beads (1). These assays are performed with mouse mAbs against HLA and anti-HLA samples from multiparous women, placentas or patients sensitized by platelet transfusions or organ transplants (2–8). The allosera are often absorbed with HLA-recombinant single antigen-expressing cell lines or with microbeads with bound single HLA antigens selected because of serological specificity of the serum sample. Absorbed antibodies are eluted from the cell line and then tested in the single allele Luminex assay. Because mAbs are considered monospecific, no absorption experiments are performed.

Terasaki's method to analyze antibody reactivity considers amino acid sequences of HLA alleles used in the Luminex assays and downloaded from the HLA Informatics area on Anthony Nolan Trust Internet Web site http://www.anthonynolan.org.uk. The antibody reactivity patterns are analyzed with a computer-based program that searches for all positively reacting alleles that exclusively share the same amino acids at sequence positions exposed on the HLA molecular surface and within the antibody-binding span estimated between about 500 and 750 $Å^2$ (9, 10). These searches consider one, two, three or four common unique amino acid positions. Amino acids that are exclusively unique to a group of alleles reacting with a mouse mAb or an eluted alloantibody are considered a distinguishing characteristic of the epitope. Studies by Terasaki's group have thus far yielded a total of 103 amino acid-defined epitopes on class I antigens encoded by HLA-A, -B and -C (4).

The HLAMatchmaker strategy of determining the HLA epitope repertoire is based on stereochemical modeling of crystallized complexes of antibodies with different protein antigens (such as hen egg lysosome and horse cytochrome c) and published data about the contributions of critical amino acid residues to antigen–antibody binding energy (11). Antigenic proteins have structural epitopes consisting of 15–22 residues that bind to the so-called paratope of antibody (12). A structural epitope has a surface area between 700 and 900 $Å^2$, and the paratope consists of six complementarity-determining regions (CDRs), three of them are on the heavy chain and the other three are on the light chain of antibody. Most structural epitopes have one patch of about two to five so-called highly energetic residues (often referred to as 'hot spots') that dominate the strength and specificity of binding with antibody (13–16). The residues of such functional epitopes are about 3 Å apart from each other, and at least one of them is nonself. Often enough, functional epitopes bind to the CDR3 of the heavy chain, which has the highest sequence variability among CDRs and plays an important role in determining antibody specificity. Certain structural epitopes have a second patch of energetic residues that contribute significantly to binding with antibody and this involves another CDR. The remaining residues of a structural epitope contribute supplementary interactions that increase the stability of the antigen–antibody complex. We have applied these concepts in the design of the 'eplet' version of HLAMatchmaker (11).

Class I HLA molecules have 75 polymorphic positions in antibody-accessible locations on the molecular surface. A determination of their residue compositions within a 3 Å radius has yielded a total of 199 so-called eplets on HLA-A, -B and -C antigens; 110 are on the α-helices, 60 are on the side surface and 29 are in less accessible positions at the bottom and under the peptide-binding groove (11). This collection of eplets represents the basis of the repertoire of HLA class I epitopes and provides a detailed assessment of HLA compatibility at the structural level. HLAMatchmaker represents a theoretical model for HLA epitope structure; a dedicated Web site http://HLAMatchmaker.net has more information. This algorithm is a clinically useful tool for analyzing antibody specificities of sera from sensitized patients and the determination of HLA mismatch acceptability (11, 17–36).

We have conducted a comparative analysis to determine how Terasaki's epitopes (TerEps) correspond to HLA-Matchmaker-defined eplets. This report shows the results for 103 HLA class I epitopes encoded by HLA-A, -B and -C (4). An accompanying paper describes our experience with class II epitopes encoded by HLA-DR and -DQ (Marrari M., Duquesnoy R., Submitted).

Methods

TerEps are described by amino acid residues shared between alleles that react with mouse mAb or human alloantibody (aAb). Most aAbs are eluates from single allele-absorbed allosera. These epitopes have identification numbers (annotated by us with #) assigned on the basis of the number of unique amino acid sites involved (#1–200 for one residue, #201–400 for two residues or #401–600 for three or four amino acid positions). TerEps have amino acid descriptions with the following notation system. Combinations of two or more residues are separated by a + sign, i.e. 56R+73T; hidden residues under the molecular surface are shown between parentheses, i.e. (74D) and (70Q), and two or more possible amino acid combinations are separated by a slash, i.e. 76V+80N/73T+76V+79R. Square brackets such as [80N] and [103L+163T] indicate epitopes also shared with HLA-C antigens but not tested in the single allele assays.

For each TerEp, we searched HLAMatchmaker for one or more eplets present on the same group of antigens and/or alleles of the Luminex panel and with similar amino acid residue compositions. This analysis consisted of four steps. First, we searched for TerEps that correspond to single eplets with comparable amino acid compositions.

The next step addressed TerEps that correspond to pairs of eplets in molecular surface locations separated far enough to be contacted by two different CDRs of antibody. These eplet pairs are analogous to the two-patch configurations of energetic residues identified on certain functional epitopes as previously described (11). Several investigators including ourselves have described HLA antibodies that react with epitopes defined by two separate surface configurations with polymorphic residues (37–41). The location of these eplet pairs was determined on crystallographic structures of HLA molecules downloaded from the http://www.ncbi.nlm.nih.gov/Structure Web site and viewed with the CN3D structure and sequence alignment software program (42). The CN3D program has a 'select by distance' (in Ångstroms) command that permits an assessment of the distances between residues. From the 700 to the 900 $Å^2$ surface area of a structural epitope, one can estimate that two eplets contacted by two different CDRs cannot be further apart than 15–20 Å.

We must also consider the influence of polymorphic residues in unexposed locations below the molecular surface. Although such hidden residues cannot make direct contact with antibody, they may alter the conformation of a nearby epitope. Several reports have described a conformational effect of hidden residues on HLA epitope reactivity with antibody (43–47). Current definitions of eplets include hidden residues within a radius of about 3 Å, but it is also possible that hidden residues somewhat further away have an effect. Therefore, eplet pairing also includes hidden residues up to 6 Å away from an eplet. Hidden residues further away are not considered to have a significant influence on eplet conformation.

The third step was to search for TerEps that correspond to polymorphic residue combinations not used so far in HLAMatchmaker. It is possible that two or more eplets in the same molecular location define the same epitope because their amino acid differences do not significantly influence the binding with specific antibody. Such eplet configurations have dominant residues that can be visualized on crystallographic structures of HLA molecules downloaded from the http://www.ncbi.nlm.nih.gov/Structure Web site and viewed with the CN3D structure and sequence alignment software program (42)

Finally, we identified a fourth group of TerEps for which we could not determine an HLAMatchmaker-defined equivalent for various reasons including that some TerEps represent xeno-epitopes rather than alloepitopes.

Results

TerEps with equivalent eplets

We have identified 50 TerEps with equivalent eplets shared by antibody-reactive alleles, 13 of them are defined by mouse mAbs and 37 by human aAbs (Table 1). Each TerEp has a list of specific antibody-reactive alleles and an amino acid description reported by Terasaki's group. Most TerEps are equivalent to single eplets, although some of them correspond to two or more eplets shared by the same allele(s). Figure 1 visualizes the locations of these eplets on molecular models of nine crystallized HLA class I antigens. For instance, six TerEps are on A*0201 (Figure 1A,B); #201 is unique for A2, and the other TerEps are shared with other antigens mostly in well-known cross-reacting groups. TerEp #17 on A2, B57 and B58 is equivalent to the 62GE eplet, which has a very similar molecular configuration on A*0201 (Figure 1A) and B*5701 (Figure 1E), whereas the 127K eplet corresponding to #19 on A2, A23, A24, A68 and A69 has the same structure on A*0201 (Figure 1A) and A*2402 (Figure1D). A side view of A2 shows that 253Q (or #38) is located on the bottom of the α3 domain (Figure 1B). TerEp #18 on A2, A68 and A69 corresponds to two closely located eplets 142MT and 145KHA in the α2 domain (Figure 1A,B).

Many TerEps are described by multiple amino acid combinations, but they correspond to single eplets. For instance, the A2-specific #201 has four possible amino acid pairs, but one eplet 66RKH describes this epitope. The A11-specific #404 has an even more complex amino acid description, but 151AHA on the α2 helix describes this epitope. Figure 1C depicts the location of 151AHA and three other eplets on A*1101: 144KR (#208), 163RW (#209) and 90D (#16). No HLA-C antigens are listed for #209, but the corresponding 90D eplet is also shared by Cw4, Cw7 and Cw18.

Table 1 has five TerEps on A24, and Figure 1D shows their locations on A*2402. A comparison between #3 (on A23 and A24) and #28 (on A23, A24 and A80) shows two closely adjoining eplets 65GKA and 62EE. Two Bw4-associated TerEps on A23 and A24 are different in #24, but not #23, is on B13, B*2705, B37, B44 and B47. These TerEps correspond to eplets 82LR and 79RI, respectively, that are only about 3 Å apart. Figure 1D–G show their locations on A*2402, B*5701, B*5101 and B*2705, respectively. Interestingly, B13, B*2705, B37, B44 and B47 share a unique eplet 79RT that is equivalent to TerEp #217 described by four possible amino acid pairs (Figure 1G). These findings show that 79RT and 79RI are mutually exclusive epitopes in the same sequence position and divide the Bw4-associated antigens according to a dominance of residues 80T and 80I.

Some TerEps might have originally been defined with Luminex kits consisting of only HLA-A and -B alleles. Their structural definitions included polymorphic residues

Table 1 Fifty TerEps that are equivalent to eplets

Ter Ep	Defined by	Antibody-reactive antigens	Residue description of TerEp[a]	Eplet(s)[b]	Models
#2	mAb	A2, 69	107W	107W	Figure 1A,B
#17	aAb	A2; B57, 58	62G	62GE	Figure 1A,B,E
#18	aAb	A2, 68, 69	142T/145H	142MT/145KHA	Figure 1A,B
#19	aAb	A2, 23, 24, 68, 69	127K	127K	Figure 1A,B,D
#201	mAb	A2	43Q+62G/62G+66K/62G+76V/62G+79G	66RKH	Figure 1A,B
#38	aAb	A2, 25, 26, 29, 31, 32, 33, 34, 43, 66, 68, 69, 74; B73; Cw7, w17	253Q	253Q	Figure 1A,B
#404	aAb	A11	149A+150A+163R/149A+158A+163R/149A+163R+166E/149A+163R+167W	151AHA	Figure 1C
#208	aAb	A1, 3, 11, 24, 36, 80	142I+144K/144K+145R	144KR	Figure 1C
#209	aAb	A11, 25, 26, 43, 6601	163R+166E/158A+163R/163R+167W	163RW	Figure 1C
#16	aAb	A1, 36, 11, 25, 26, 34, 43, 6601, 80; B73	[90D]	90D (also on Cw4, 6, 7, 18)	Figure 1C
#3	mAb	A23, 24	65G	65GKA	Figure 1D
#28	mAb	A23, 24, 80	62E	62EE	Figure 1D
#14	aAb	A1, 23, 2402, 80; B76	166D/167G	167DG	Figure 1D
#24	aAb	A23, 24, 25, 32; B13, 2705, 37, 38, 44, 47, 49, 51, 52, 53, 57, 58, 59, 63, 77	82L/83R	82LR	Figure 1D–G
#23	aAb	A23, 24, 25, 32; B38, 49, 51, 52, 53, 57, 58, 59, 63, 77	80I	79RI	Figure 1D,E,F
#217	aAb	B13, 2705, 37, 44, 47	76E+80T/79R+80T/80T+82L/80T+83R	79RT	Figure 1G
#207	mAb	B57, 58, 63	43P+65R/65R+163L/66N+131S/66N+163L	71SA	Figure 1E
#245	aAb	B35, 4005, 46, 49, 50, 51, 52, 53, 56, 57, 58, 62, 63, 71, 72, 75, 77, 78; Cw9, w10	163L+167W	163LW	Figure 1E,F,I
#35	mAb	B18, 35, 37, 51, 52, 53, 58, 78	45T	44RT	Figure 1F
#222	aAb	A6602; 7, 13, 27, 47, 48, 60, 61, 73, 81; Cw2, w17	163E+166E/163E+167W	163EW	Figure 1G
#229	aAb	B7, 27, 42, 54, 55, 56, 67, 73, 81, 82	65Q+69A	65QIA	Figure 1G
#20	aAb	B7, 8, 41, 42, 48, 60, 81	177D/180E	180E	Figure 1H
#25	aAb	B7, 8, 18, 2708, 35, 39, 4005, 41, 42, 45, 46, 48, 50, 54, 55, 56, 60, 61, 62, 64, 65, 67, 71, 72, 73, 75, 76, 78, 81, 82	[80N]	79RN (also on Cw1, 3, 7, 8, 12, 14, 16)	Figure 1H
#216	aAb	B7, 8, 18, 2708, 35, 39, 4005, 41, 42, 45, 48, 50, 54, 55, 56, 60, 61, 62, 64, 65, 67, 71, 72, 75, 76, 78, 81, 82	76E+80N/76E+82R/76E+83G	80ERN	Figure 1H
#22	aAb	B8, 13, 18, 35, 37, 38, 39, 3905, 4005, 41, 44, 45, 47, 48, 49, 50, 51, 52, 53, 59, 60, 61, 62, 64, 65, 71, 72, 75, 76, 77, 78	69T	71NT	Figure 1H

Table 1 *Continued*

Ter Ep	Defined by	Antibody-reactive antigens	Residue description of TerEp[a]	Eplet(s)[b]	Models
#39	aAb	Cw2, w9, w10, w15	21H	21H	Figure 1I
#246	aAb	B46, 73; Cw1, w7, w8, w9, w10, w12, w14, w16	76V+80N/73T+76V+79R	80VRN	Figure 1I
#421	aAb	B46; Cw1, w8, w9, w10, w14, w16	(73T)+76V+80N+90A	77TVS	Figure 1I
#1	mAb	A1, 36	44K/150V/158V	44KM/152HA/158V	
#6	mAb	A3	161D	161D	
#4	mAb	A25, 26, 34, 43, 66	(9Y)+149T/(74D)+149T	145QRT	
#30	aAb	A1102	19K	19K	
#5	mAb	A29, 43	62L	62LQ	
#36	aAb	A30	17S/56R+73T	17RS	
#31	aAb	A30, 31	56R	56R	
#29	aAb	A80	56E/62E+65R/62E+76A/144K+151R/163E+166D/163E+167G	56E/163EG	
#408	mAb	B7	(147W)+163E+177D/(147W)+163E+180E	177DK	B*0702 only
#8	mAb	B13	145L/41T+46A	144QL	
#9	mAb	B38, 39, 67	158T	158T	
#240	aAb	B76	163L+166D/163L+167G	163LG	
#37	aAb	Cw7	194L	193PL/267MQ/273SE	
#40	aAb	Cw5, w8	177K	46QGE/177KT	
#41	aAb	B73; Cw7, w17	267Q	267QE	
#402	aAb	B7, 42, 54, 55, 56, 67, 81, 82	65Q+69A+(70Q)	70IAQ	#402 = #410
#410	aAb	B7, 42, 54, 55, 56, 67, 81, 82	[41A+46E+67Y/43P+46E+67Y/43P+70Q+76E/43P+46E+70Q]/43P+69A+70Q/[46E+65Q+67Y/46E+65Q+70Q]	70IAQ	#402 = #410
#244	aAb	Cw2, w4, w5, w6, w15, w17, w18	77N+80K	79RK	
#15	aAb	A1, 26, 29, 36, 43, 80	76A	76ANT	
#21	aAb	B13, 4005, 41, 44, 45, 47, 49, 50, 60, 61	41T	41T	
#27	aAb	A203, 25, 26, 34, 43, 66	149T	150TAH	
#211	aAb	A203, 25, 26, 34, 43, 66, B46, 62, 76	[(152E+156W)]	158WA	Also on Cw2, w6, w12

aAb, alloantibody generally eluted from antigen used to absorb alloserum; mAb, monoclonal antibody; TerEps, Terasaki's epitopes.
[a] Amino acids in HLA protein sequence positions are listed with the standard single letter code. Amino acids not exposed on surface of molecule are in parenthesis. Residues shared with C locus antigens but not proven by single allele antibody testing are indicated as square brackets. TerEps described by combinations of residues are shown with + sign. Possible alternative residue combinations are separated by slash.
[b] Two and three unique eplets are separated by '/'.

also found on HLA-C antigens; they are shown between square brackets. For instance, #25 on a group of Bw6-associated antigens is described by [80N], but the corresponding eplet 79RN is also on a group of HLA-C antigens. Interestingly, #216 is on the same group of antigens except B46, B73 and any HLA-C antigens. This TerEp corresponds to 80ERN that shares the 79R and 80N residues with 79RN (Figure 1H). Figure 1I shows four eplet-defined TerEps on Cw*0303. TerEps #246 and #421 are on the same group of HLA-B and -C antigens except B73, Cw7 and Cw12 that are only on #246; they correspond to 80VRN and 77TVS, respectively.

No crystallized HLA antigen structures are available for showing the locations of the remaining eplets in Table 1. Almost all of them correspond to single eplets. Four TerEps correspond to two or three possible eplets, namely #1 on A1 and A36: 44KM/152HA/158V, #29 on A80: 56E/163EG/267KE, #37 on Cw7: 193PL/267MQ/273SE (all are in the α3 domain) and #40 on Cw5 and Cw8: 46QGE/177KT.

Some TerEps' descriptions include residues below the molecular surface, but they seem equivalent to eplets that do not require hidden residues. As an example, #4 on A25, A26, A34, A43 and A66 is described by (9Y)+149T/(74D)+149T. The hidden 9Y and 74D residues are more

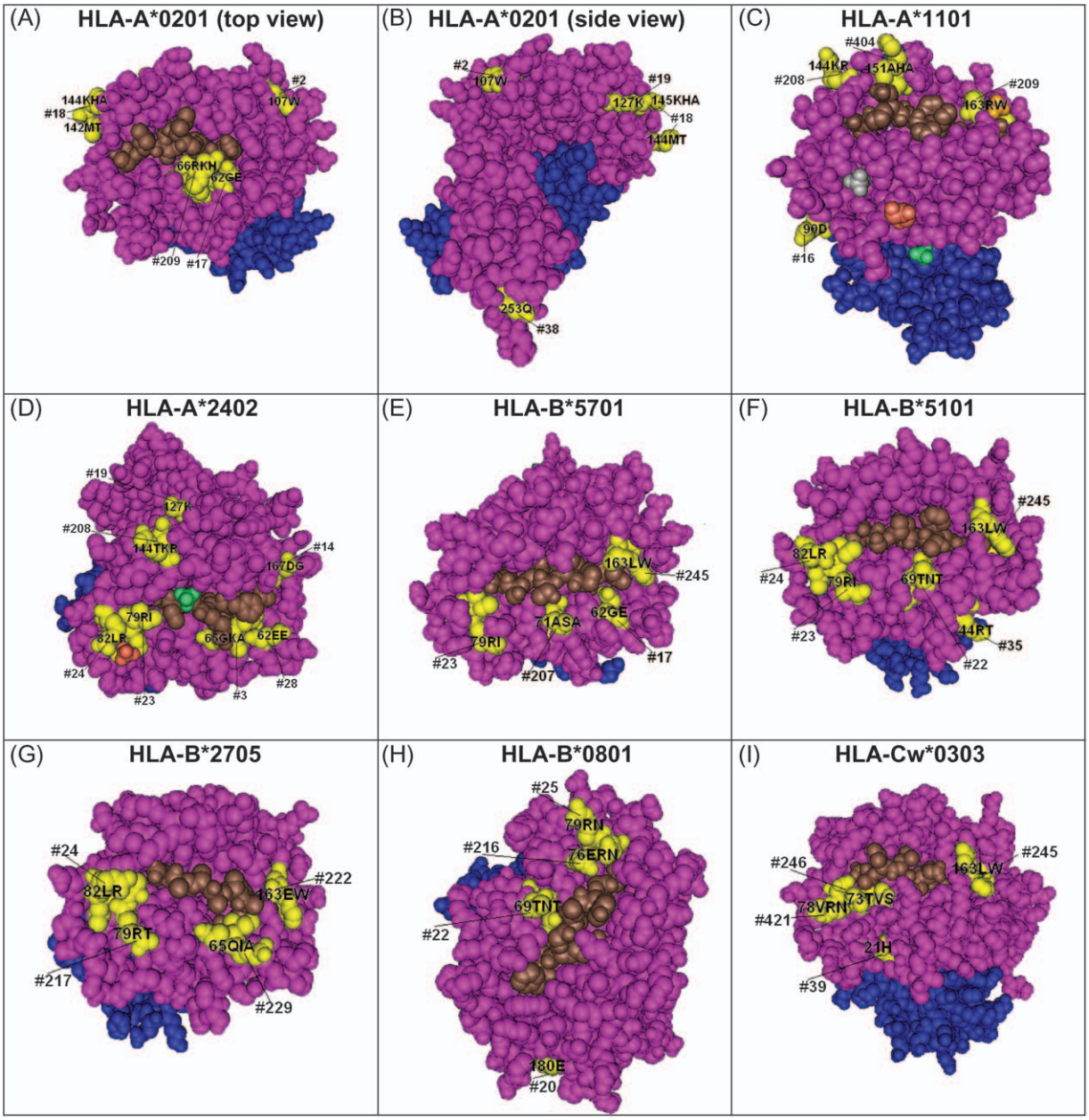

Figure 1 Locations of Terasaki's epitopes and their equivalent eplets on HLA molecules (color codes: eplet residues are in yellow, α-chain in magenta, β2-microglobulin in blue and peptide in green).

than 18 Å away from 149T, too far for any conformational influence on a 149T-defined epitope. Our analysis suggests that #4 is equivalent to the 145QRT eplet. Moreover, the hidden 152E and 156W describe #211, but neither of them can make direct contact with antibody. We conclude that #211 most likely corresponds to 158WA because 156W and the surface-expressed 158A are only 3.3 Å apart.

In one case, identical groups of antigens in the Luminex panel share a pair of TerEps. Both #402 and #410 are on the B7, B42, B54, B55, B56, B67, B81 and B82 groups and are equivalent to 70IAQ.

TerEps corresponding to eplet pairs

This analysis has shown that about one-half of TerEps correspond to single eplets. The next step is to search for TerEps that correspond to pairs of eplets in locations sufficiently away from each other for contact by two different CDRs of antibody. Previous studies on human mAbs have shown specificity patterns against a combination of nonself and self amino acid triplets that defined the epitopes (40). For instance, the reactivity of a 62QE-specific antibody required the presence of a glycine residue in position 56 found on the immunizing HLA-A3 molecule.

Twenty TerEps are equivalent to an eplet pair, and 11 TerEps correspond to two or more possible eplet pairs (Table 2). We have determined the locations of eplet pairs on the molecular surface when a structural model of an informative HLA antigen was available (Figure 2).

Two TerEps correspond to 79RI paired with another eplet. A comparison between #23 with its 79RI equivalent (Table 1) and #212 shows that both TerEps are on the same group of Bw4-associated antigens except for A25, which does not have #212. Apparently, #212 corresponds to 79RI but requires another structural configuration that distinguishes A25 from the other Bw4-associated antigens. The only possibility is position 90, whereby A25 has 90D rather than 90A. Thus, #212 corresponds to 79RI+90A, and Figure 2A shows the locations of these eplets on B*5101; they are about 11 Å apart. Although #419 on the Bw4-associated B49, B51, B52, B63 and B77 has a very complex amino acid description, we could readily identify 79RI+152RE as the corresponding eplet pair. Figure 2B shows the locations of these eplet pairs on B*5101; they are about 15 Å apart. TerEp #230 is on another subgroup of Bw4-associated antigens: B38, B49, B51, B52, B53, B59 and B77. Its description with eight amino acids seems very complex, but two corresponding eplet pairs are possible: 65QIT+79RI and 71NT+79RI. Figure 2C shows that 65QIT and 71NT are close together; they may constitute a single contact site for one CDR, whereas 79RI would contact another CDR of the #230-specific antibody.

HLA-B18, -B35, -B37, -B51, -B52, -B53, -B58 and -B78 share #35, which is equivalent to 44RT (Table 1 and Figure 1F). TerEp #219 is on the same group of antigens except B58, which has 71SA rather than 71NT shared between the other antigens. Therefore, #219 corresponds to 44RT+71NT. Figure 2D shows the locations of these eplets on B*5101; they are about 11 Å apart. TerEp #403 on B46, B62, B75, B76 and B77 is equivalent to 45RMA+79RN (Figure 2E).

Table 1 shows that a large group of antigens express #245 and its equivalent eplet 163LW. Two TerEps, namely #221 and #415 on different subgroups, correspond to 163LW paired with 131S and 71AT, respectively. Figure 2F,G shows the locations of these eplets on B*5701. Although none of the TerEps corresponded fully to 163TW, we identified five TerEps that are equivalent to pairs involving this eplet, namely #204 is 109L+163TW (Figure 2H), #228 is 131S+163TW (no figure), #215 is 62RN+163TW (Figure 2I), #232 is 103L+163TW (Figure 2J) and #225 is 66QIF+163TW (Figure 2K). The amino acid descriptions of #204 and #415 are between square brackets, i.e. they may also be on HLA-C antigens. The 109L+163TW equivalent of #204 is on Cw1, Cw4, Cw5, Cw6, Cw8, Cw12, Cw14, Cw15, Cw16 and Cw18. TerEp #415 on B46, B57, B58 and B63 with its 71AT+163LW equivalent may also be on Cw9 and Cw10.

Two TerEps correspond to eplets that appear under the influence of nearby hidden residues. B8, B64 and B65 share #420 that corresponds to combination of three eplets, 71NT(h)+158A+163TW, whereby (h) indicates three hidden residues 25S, 74D and 95L nearby 71NT. As shown in Figure 2M, 158A and 163TW display a linear configuration that may serve as a contact site for a CDR loop of the #420-specific antibody. The B8-specific #11 is described by two hidden residues 67F and 9D and two surface residues 131R and 181E that are about 20 Å away, too far for any interactions that may lead to a distinct epitope. Our best estimate is a pair of nearby eplets, both of which may have conformational influences by hidden residues. Figure 2N shows 66QIF(h)+71NT(h) on B*0801, whereby (h) represents the complex of hidden residues 9D, 24S, 67F, 74D and 95L.

Table 2 shows four TerEps corresponding to eplets paired with a locus-specific monomorphic residue: #214 is 62RN+m43Q (Figure 2L), #241 is 90D+m138M found on the side of the molecule (Figure 2O), #239 is 80VRN+m43P and #213 is 144QR+m138M. All HLA-A but no HLA-B antigens have m43Q and m138M, and all HLA-B but no HLA-A antigens have m43P. These epitopes are equivalent to 'locus-restricted' eplets.

TerEp #220 on A*3301, B18, B51, B52, B64, B65 and B78 is described by 90A and (171H), which is within 3 Å from 166EW. Our previous triplet notation has171H (48), which is the same as 166EWH. Although #220 corresponds to 166EWH, we noted that this eplet is also on B73 that has 90D instead of 90A. Being >35 Å away, this residue is too far for pairing with 166EWH. A better choice would be 103V (B73 has 103M) that is about 9 Å away. Thus, #220 may correspond to 166EWH+103V.

Table 2 lists seven TerEps that are unique to a single antigen not commonly defined by serology. Two TerEps #10 (on B46) and #203 (on A2403) are equivalent to a single eplet pair, namely 45RM+71QA and 152HV+163TG, respectively. Five TerEps (#202 on A23, #407 on A24, #206 on A36, #406 on B*2705 and #411 on B*2708) have rather complex amino acid descriptions and correspond to two or more possible eplet pairs. It should be noted that mouse mAbs define six of these seven TerEps. Because monospecific aAbs against these TerEps are extremely rare, it is possible that they have little clinical relevance regarding humoral allosensitization.

TerEps defined by dominant residues on eplets

Altogether, 81 of 103 TerEps are equivalent to HLA-Matchmaker-defined epitopes represented by single eplets or eplet pairs. For the remaining TerEps, we have searched for eplets with amino acid variations that may permit a structural type of cross-reactivity. In each case, we determined with the CN3D viewer all residues within a 3 Å

Table 2 Thirty-one TerEps that correspond to eplet pairs

TerEp	Defined by	Antibody-reactive antigens	Residue description of TerEp	Eplet pair(s)	Models
#212	aAb	A23, 24, 32; B38, 49, 51, 52, 53, 57, 58, 59, 63, 77	[80I+90A/80I+149A]	79RI+90A	Figure 2A
#419	mAb	B49, 51, 52, 63, 77	80I+90A+127N+(152E)/80I+109L+131S+(152E)/82L+90A+127N+(152E)/83R+90A+127N+(152E)	79RI+152RE	Figure 2B
#230	aAb	B38, 49, 51, 52, 53, 59, 77	65Q+80I/69T+80I	65QIT+79RI/71NT+79RI	Figure 2C
#219	aAb	B18, 35, 37, 51, 52, 53, 78	45T+62R/45T+65Q/45T+66I/45T+69T/45T+71T	44RT+71NT	Figure 2D
#403	aAb	B46, 62, 75, 76, 77	41A+46A+65Q	45RMA+79RN	Figure 2E
#221	aAb	B35, 4005, 46, 49, 50, 51, 52, 53, 56, 57, 58, 62, 63, 71, 72, 75, 77, 78	163L+167W	131S+163LW	Figure 2F
#415	mAb	B46, 57, 58, 63	[(63E)+(71A)+163L]	71AT+163LW (also on Cw9, 10)	Figure 2G
#204	mAb	A32, 74; B8, 18, 37, 38, 39, 41, 42, 54, 55, 59, 64, 65, 67	[109L+163T]	109L+163TW (also on Cw1, 4, 5, 6, 8, 12, 14, 15, 16, 18)	Figure 2H
#228	aAb	B18, 37, 38, 39, 54, 55, 56, 64, 65, 67	131S+163T	131S+163TW	
#215	aAb	A33, 34, 68, 69; B8, 18, 37, 38, 39, 41, 42, 54, 55, 59, 64, 65, 67	62R+163T	62RN+163TW	Figure 2I
#232	mAb	B54, 55, 59; Cw1, w4, w5, w6, w7, w8, w12, w14, w15, w16, w18	(103L)+163T	103L+163TW	Figure 2J
#225	aAb	B8, 59	(67F)+163T	66QIF+163TW	Figure 2K
#420	mAb	B8, 64, 65	69T+(74D)+158A+163T	71NT(h)[a]+163TW+158A	Figure 2M
#11	mAb	B8	(67F)+131R/(67F)+177D/(67F)+180E [9D]	66QIF(h)+71NT(h)[b]	Figure 2N
#214	aAb	A25, 26, 33, 34, 66, 68, 69	43Q+62R/62R+109F	62RN+m43Q	Figure 2L
#241	aAb	A1, 11, 25, 26, 34, 36, 43, 6601, 80	65R+90D/43Q+90D/90D+138M	90D+m138M	Figure 2O
#213	aAb	A23, 25, 26, 29, 30, 31, 32, 33, 34, 43, 66, 74	138M+144Q	144QR+m138M	
#239	aAb	B46, 73	43P+76V/65Q+76V/76V+79R/[76V+80N/41A+43P+76V [41A+65Q+76V/73T+76V+79R/73T+76V+80N]	80VRN+m43P	
#203	mAb	A2402	156Q+166D/156Q+167G	152HV+166DG	
#10	mAb	B46	46A+66K [69R]	44RM+71QA	
#237	mAb	B57, 63 (weak B58)	46A+65R/41A+46A+65R [41A+65Q+76V/73T+76V+79R/73T+76V+80N]	44RM+71SA	
#242	mAb	A1, 2, 3, 1101, 26, 29, 30, 31, 33, 34, 36, 43, 66, 68, 69, 74, 80	19E+79G	79GT+19E	
#414	aAb	B49, 52, 63	62R+(63E)+80I	62RE+76ENI	
#220	aAb	A3301; B18, 51, 52, 64, 65, 78	90A+(171H)	166EWH+103V	
#227	aAb	B8, 18, 35, 39, 4005, 41, 45, 48, 50, 60, 61, 62, 64, 65, 71, 72, 75, 76, 78	69T+80N/69T+82R/69T+83G	65QIT+73TS/65QIT+79RN/71NT+79RN	
#411	aAb	B2708	(63E)+69A+80N/(70K)+76E+80N/(70K)+76E+82R/(70K)+76E+83G/(70K)+80N+131S	71KA+80ERN/66QIK+73TS	
#202	mAb	A23	65G+151R/127K+144Q/127K+151R	65GKA+152RV/152RV+163TG	
#206	mAb	A36	158V+163T/158V+166E/158V+167W	44KM+163TVV/152HA+163TW	
#236	mAb	B57, 58	43P+62G/41A+43P+62G/17R+43P+62G/19E+41A+43P+62G	62GE+m43P/62GE+71SA/62GE+163LW/71SA+152RV	

Table 2 *Continued*

TerEp	Defined by	Antibody-reactive antigens	Residue description of TerEp	Eplet pair(s)	Models
#406	mAb	B2705	65Q+69A+80T/65Q+69A+82L/65Q+69A+83R	71KA+79RT/65QIA+ 79RT/44RE+73TD	
#407	mAb	A24	127K+142I+144K/127K+142I+151H/127K+144K+ 145R/127K+145R+151H	65GKA+152HV/152HV+ 79RI/145KRA+79RI/ 142MI+151AHV	

aAb, alloantibody generally eluted from antigen used to absorb alloserum; mAb, monoclonal antibody; TerEps, Terasaki's epitopes.
[a] Nearby hidden residues 25S, 74D and 95L are considered to affect the conformation of 71NT (Figure 2M).
[b] Nearby hidden residues 9D, 24S, 74D and 95L are considered to affect the conformation of 66QIF and 71NT (Figure 2N).

radius that form a patch with the polymorphic surface residue that describes the TerEp. Figure 3 shows the locations of the residues on informative HLA antigens. The upper part (yellow) shows the surface-exposed 3-Å patch around the polymorphic residue. The lower part depicts the patch in detail: polymorphic residues are colored yellow, permissible residue combinations are recorded next to residues colored in magenta and monomorphic residues in yellow are marked only with their sequence position number. Table 3 lists 12 TerEps that correspond to eplets with permissible residue combinations; each eplet is marked with an asterisk indicating the presence of permissible combinations.

A single residue 163R describes #12 on A1, A11, A25, A26, A43 and A*6601. The structural model of A*1101 shows five residues in a 3-Å patch around 163R: 162G (monomorphic), 163R, 165V (monomorphic), 166E and 167W (Figure 3A). All antigens carrying #12 have this amino acid combination except A1, which differs with 166D and 167G. Therefore, 166D/E and 167G/W are considered as permissible combinations because they do not affect the specificity of #12. Thus, 163R dominates this epitope, whereas the monomorphic 162G and 165V align themselves with 163R as potential contact sites for the CDR loop that determines the specificity of the #12-specific antibody. The eplet notation system does not include monomorphic residues and #12 corresponds to 162R*, whereby * represents the permissible 166D/E and 167G/W combinations.

Two closely located surface residues 62R and 65R describe #243 on A25, A26, A33, A34, A66, A68, A69 and B63. Figure 3B shows eight residues in the overlapping 3-Å patches of 62R and 65R on A*6901, namely 58E, 59Y, 61D (all monomorphic), 62R, 63N, 64T (monomorphic), 65R and 66N. All #243-carrying antigens share these residues except B63, which has 63E instead, and A34 (A*3401), which has 66K. This structural presentation illustrates the dominance of the 62R and 65R aligned for possible contact with the specificity-determining CDR loop of anti-#243 antibody, whereas 63E/N and 66K/N do not seem to have a significant effect on this epitope. The B63-specific #409 corresponds to a locus-restricted eplet pair 62RR*+m43P, whereby * represents 63E/N and 66K/N and m43P is a monomorphic residue on HLA-B but not on HLA-A.

The 144K surface residue describes #13 on A1, A2, A3, A11, A24, A36, A68, A69 and A80. The 3-Å patch of 144K on A*1101 consists of five residues: 140A (monomorphic), 143T, 144K, 145R and 148E (monomorphic) (Figure 3C). All #13-carrying residues have this residue combination except A2, A68 and A69, which have 145H instead of 145R. Although #13 corresponds to 143TK*, whereby * is 145H/R; it is possible that 144K aligned with the monomorphic 148E dominates this epitope. Interestingly, two TerEps in Table 1 distinguish 145R from 145H in the 3-Å patch of 144K, namely #208 equivalent to 144KR and #18 on A2, A68 and A69 that corresponds to 145KHA. TerEp #210 on A2, A3, A11, A68 and A69 corresponds to 144K* paired with 76VD (Table 3 and Figure 3D). These findings illustrate how a 3-Å patch of 144K can generate different epitopes such as 144K* (#13), 144KR (#208), 145KHA (#18) and 144K*+76VD (#210).

TerEps #223 and #235 are on the so-called B7 cross-reacting group of antigens, namely B7, B13, B27, B47, B48, B60, B61 and B81, but #235 is also on B73. They correspond to 76ER*+163EW, whereby * is 77E/N/S and 80N/T (Figure 3E), and to 65QI*+163EW, whereby * is 62N/R, 67C/S/V and 70K/N/Q (Figure 3F). TerEp #226 is on another group of HLA-B antigens and corresponds to 62RQI*+163TW, whereby * is 67C/F/S/Y and 70N/Q (Figure 3G). A large group of HLA-A and -B antigens has #238, which corresponds to 65RA*+56G, whereby * is 66K/N and 67M/V. Figure 3H,I shows that they have very similar structural configurations on A*0201 and B*5701. TerEp #218 is equivalent to 76E*+45RMA, whereby * is 77N/S and 80N/T (Figure 3J). These models illustrate how polymorphic residues align themselves to dominate these epitopes, i.e. 76E+79R of #223 (Figure 3E), 65Q+66I of #235 (Figure 3F), 62R+65Q+66I (Figure 3G) 65R+69A of #238 (Figure 3H,I) and 76E (Figure 3J). These contiguous alignments, which may consist of discontinuous sequences and may include monomorphic residues, seem suitable contact areas for the linear sequences of CDR loops that mediate antibody specificity.

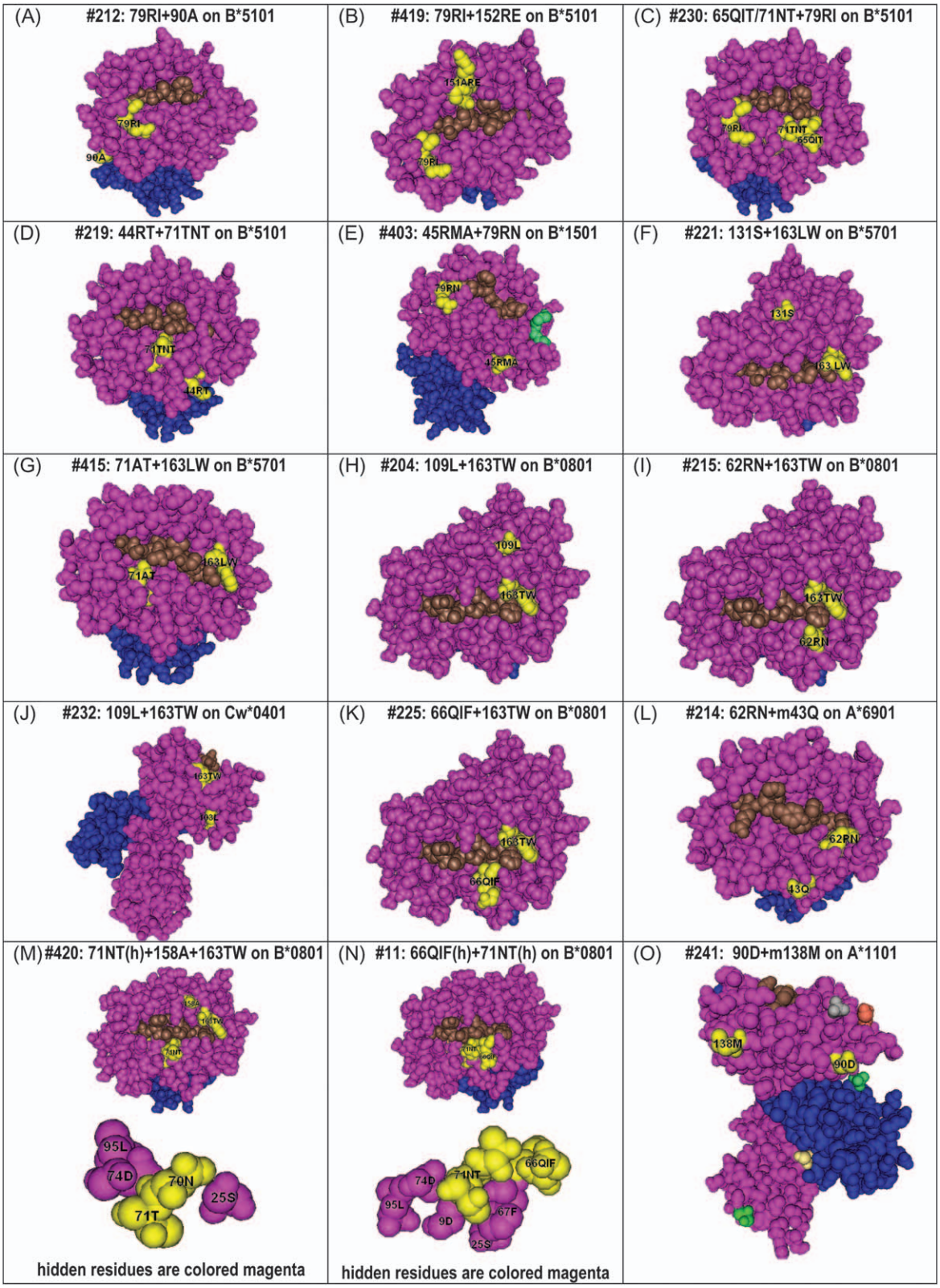

Figure 2 Locations of Terasaki's epitopes and their equivalent eplet pairs (color codes: see Figure 1).

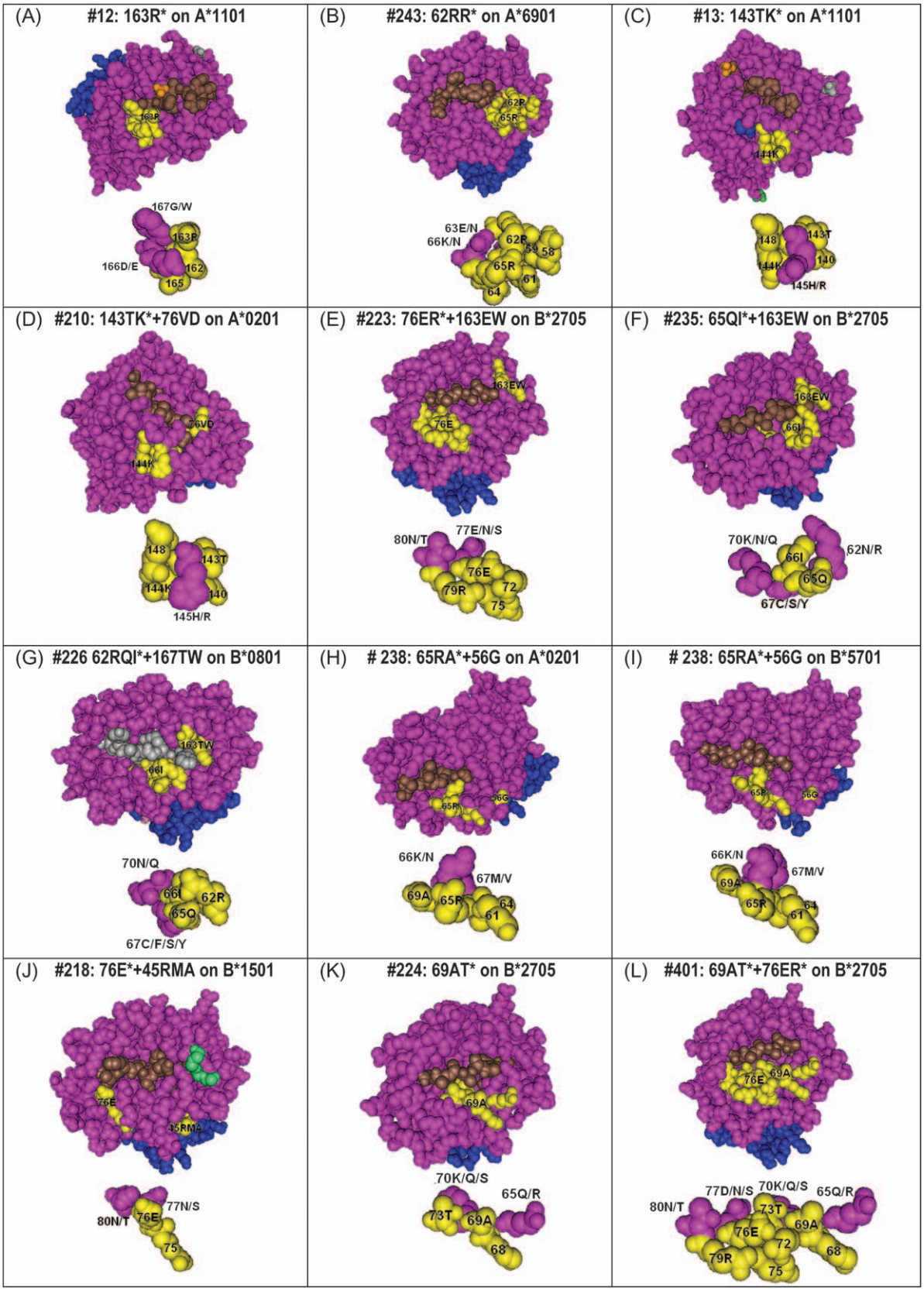

Figure 3 Terasaki's epitopes equivalent to eplets with permissible amino acid combinations (color codes: see Figure 1).

Table 3 Twelve TerEps that correspond to eplets with permissible residue substitutions

TerEp	Defined by	Antibody-reactive antigens	Residue description of TerEp	Eplets	Permissible substitutions	Models
#12	aAb	A1, 11, 25, 26, 43, 6601	163R	163R*	166D/E; 167G/W	Figure 3A
#243	mAb	A25, 26, 33, 34, 66, 68, 69; B63	62R+65R	62RR*	63E/N; 66K/N	Figure 3B
#409	mAb	B63	43P+62R+65R	62RR*+m43P	63E/N; 66K/N	
#13	aAb	A1, 2, 3, 11, 24, 36, 68, 69, 80	144K	143TK*	145H/R	Figure 3C
#210	aAb	A2, 3, 11, 68, 69	76V+144K	143TK*+76VD	145H/R	Figure 3D
#223	aAb	B7, 13, 27, 47, 48, 60, 61, 81	76E+163E	76ER*+163EW	77E/N/S; 80N/T	Figure 3E
#235	mAb	B7, 13, 27, 47, 48, 60, 61, 73, 81	138T+163E	65QI*+163EW	62N/R; 67C/S/V; 70K/N/Q	Figure 3F
#226	aAb	B8, 18, 37, 38, 39, 41, 42, 54, 55, 59, 64, 65, 67	661+163T	62RQI*+163TW	67C/F/S/Y; 70N/Q	Figure 3G
#238	mAb	A1, 2, 3, 11, 25, 26, 29, 32, 33, 34, 36, 43, 66, 68, 69, 74; B57, 58, 63	56G+65R	65RA*+56G	66K/N; 67M/V	Figure 3H,I
#218	aAb	B13, 57, 62, 63, 75, 76, 77	46A+76E	76E*+45RMA	77N/S; 80NT	Figure 3J
#224	aAb	B7, 27, 42, 54, 55, 56, 57, 58, 63, 67, 73, 81, 82	43P+69A	69AT*	65Q/R; 70K/Q/S	Figure 3K
#401	aAb	B7, 27, 42, 54, 55, 56, 57, 58, 63, 67, 81, 82	43P+69A+76E	69AT*+76ER*	77D/N/S; 80N/T	Figure 3L

aAb, alloantibody generally eluted from antigen used to absorb alloserum; mAb, monoclonal antibody; TerEps, Terasaki's epitopes.

Two TerEps share the same group of HLA-B antigens except B73. TerEp #224 corresponds to 69AT*, whereby * is 65Q/R and 70K/Q/S (Figure 3K). TerEp #401 is equivalent to a pair of overlapping eplets with permissible residues: 69AT*, whereby * is 65Q/R and 70K/Q/S, and 76ER*, whereby * is 77D/N/S and 80N/T. Figure 3L illustrates how the alignment of 79R, 76E, 73T, 69A and the monomorphic 68K may represent the contact areas for two CDR loops of antibody.

TerEps without corresponding eplets

We could not find corresponding eplet descriptions for 10 TerEps, 8 of which are defined by mouse mAbs (Table 4). The amino acid configurations of #7, #32, #33, #205, #233 and #234 are monomorphic for HLA-B and/or -C. As an example, the two possible amino acid descriptions [43P+(70Q)/65Q+(70Q)] of #234 are on all HLA-C antigens and cannot describe this alloepitope. Moreover, the hidden 70Q is almost 15 Å from 43P (monomorphic for HLA-B and -C), too far for any conformational interaction. These xeno-epitopes consist of exclusively self-residues that cannot elicit aAbs in humans and therefore are excluded from HLAMatchmaker.

Four remaining TerEps in Table 4 have complex descriptions of exposed and hidden residues, and we could not identify corresponding eplets on antibody-reactive antigens. A*0201 has TerEp#412, which is defined by two alternative pairs 142T+149A/145H+149A plus the hidden residue 9F. This residue can distinguish A*0201 from A*0206, but it cannot make direct contact with antibody nor would it have a significant conformational influence because it is too far away (almost 20 Å) from 142T+149A/145H+149A. Although the 150TAH eplet permits an easy distinction of A*0203 from A*0201 and A*0206 in the Luminex panel, it has remained very difficult to differentiate between A*0201 and A*0206.

The #417 xeno-epitope reacts with A11, B57 and B58 and correlates with the surface-exposed 41A plus three hidden residues 9Y, 63E and 95I. The latter cannot make direct contact with antibody, and with a distance of more than 15 Å, they seem too far away to have a conformational effect on 41A. TerEp #418 is on A26 and B13, which share 62R+80T plus the hidden residues 32Q, 45M and 77N. Because the surface residues 62R and 80T are about 23 Å away, they can be contacted at best by two separate CDRs of antibody. 77N is sufficiently close for a conformational effect on 80T whereas 32Q and 45M are 11 and 6 Å away. Although these hidden residues seem necessary to define #418, it is not understood why surface residue differences between A26 and B13 around residue 62R (NRN *vs* EQY) and around 80T (GLRG *vs* RALR) would have no effect on the specificity of this epitope. Therefore, the composition of this TerEp needs further clarification.

The 41A+178K description of the mAb-defined #231 on B7, B48 and B81 is confusing: 41A is monomorphic for all class I loci and 178K is only on B*0702. The best eplet description of #231 is the pair of 163EW+76ESN with a conformational effect of the hidden 9Y, 11S and 12V residues that are about 6 Å from 76ESN. Another possibility is 163EW+180E, but this pair is also found on B60.

Eplets without equivalent TerEps

This analysis has shown that 93/103 TerEps correspond to eplet configurations, but they do not represent all alloepitopes relevant to humoral allosensitization. HLAMatchmaker

Table 4 List of TerEps for which no corresponding eplet could be identified

TerEp	Defined by	Antibody-reactive antigens	Residue description of TerEp	Comments
#7	mAb	B7, 8, 13, 18, 27, 35, 37, 38, 39, 4005, 41, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 59, 60, 61, 62, 64, 65, 67, 71, 72, 73, 75, 76, 77, 78, 81, 82	65Q	Monomorphic for HLA-C
#32	mAb	A2, 3, 11, 2403, 25, 26, 29, 30, 31, 32, 33, 34, 36, 43, 66, 68, 69, 74; B7, 8, 13, 18, 27, 35, 37, 38, 39, 4005, 41, 42, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 67, 71, 72, 73, 75, 77, 78, 81; Cw1, w2, w4, w5, w6, w7, w8, w9, w10, w12, w14, w15, w16, w17, w18	167W	Monomorphic for HLA-C
#33	mAb	A32, 74; B7, 8, 13, 18, 27, 35, 37, 38, 39, 4005, 41, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 67, 71, 72, 73, 75, 76, 77, 78, 81, 82	[109L]	Monomorphic for HLA-B and -C
#205	mAb	A32 74; B7, 8, 4005, 41, 42, 48, 60, 61, 73, 81; Cw1, w2, w4, w5, w6, w7, w8, w9, w10, w12, w14, w15, w16, w17, w18	109L+131R	Monomorphic for HLA-C
#233	mAb	A25, 32; B7, 8, 13, 18, 27, 35, 37, 38, 39, 4005, 41, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64,65, 67, 71, 72, 73, 75, 76, 77, 78, 81, 82	[79R+127N]	Monomorphic for HLA-B and -C
#234	aAb	B7, 42, 46, 54, 55, 56, 67, 81, 82	[43P+(70Q)/65Q+(70Q)]	Monomorphic for HLA-C
#412	aAb	A0201	(9F)+142T+149A/(9F)+145H+149A	No identifiable eplet
#417	mAb	A11; B57, 58	[(9Y)+41A+(63E)+(95I)]	No identifiable eplet
#418	mAb	A26; B13	(32Q)+62R+(77N)+80T/(45M)+62R+(77N)+80T	No identifiable eplet
#231	mAb	B7, 48, 81	41A+178K	No identifiable eplet

aAb, alloantibody generally eluted from antigen used to absorb alloserum; mAb, monoclonal antibody; TerEps, Terasaki's epitopes.

represents a theoretical model for HLA epitope structure, and our analysis has suggested up to 199 eplets on HLA-A, -B and -C antigens (11). Table 5 lists 38 eplets in well-exposed surface positions that are not included in the current list of TerEps. Previous analyses of sera screened with HLA-typed panels have commonly identified antibodies specific for most of these eplets. Prime examples are antibodies to 44RK (on B4005, B41, B44, B45, B47, B49,

Table 5 Eplets not included in the Terasaki's epitope repertoire

Eplet	Associated antigen summary
1C.6K	Cw1
44RK	B60, 61, 4005, 41, 44, 45, 47, 49, 50
62QE	A1, 3, 11, 30, 31, 32, 36, 74
65RNA	A1, 3, 11, 25, 26, 28, 29, 30, 31, 32, 36, 43, 66, 74, 80; B63, 57, 58
66RNH	A1, 25, 26, 31, 32, 33, 36, 43, 74, 80
66RNQ	A3, 11, 29, 30, 66, 68, 69
70KAH	A2, 23, 24
71HS	A1, 2, 23, 24, 25, 26, 31, 32, 33, 36, 43, 74, 80
71QS	A3, 11, 29, 30, 34, 66, 68, 69
73AN	Cw4, w6, w17, w18
73ID	A31, 33
73TD	A2, 3, 11, 30, 34, 66, 68, 69, 74; B27, 37, 47
73TN	A1, 9, 26, 29, 36, 43, 80; B5, 13, 17, 27, 38, 44, 49, 53, 59, 63, 77; Cw2, w15
76EDT	B27, 37, 47
76ENT	B13, 44
76ESI	A25, 32
76VDT	A2, 3, 11, 30, 31, 33, 34, 66, 68, 69, 74
77TEN	A23, 24; B13, 63, 77, 27, 38, 44, 49, 51, 52, 53, 57, 58, 59
77TVN	Cw2, w5, w15, w16
78VGT	A2, 3, 11, 30, 34, 66, 68, 69, 74
105S	A2, 3, 23, 24, 29, 30, 31, 33, 68, 69, 80
109F	A1, 2, 3, 9, 11, 25, 26, 29, 30, 31, 33, 34, 36, 43, 66, 68, 69, 80
142MI	A1, 3, 11, 9, 25, 26, 29, 30, 31, 32, 33, 34, 36, 43, 66, 68, 74, 80
147L	B60, 48, 81; Cw7, w17
150AAH	A2, 3, 11, 24, 68, 69
151AHE	A*0203, 3, 25, 26, 34, 43, 66
151ARV	A23, 29, 31, 32, 33, 74; B8, 13, 16, 17, 18, 22, 27, 35, 37, 40, 41, 42, 45, 47, 48, 53, 59, 67, 73, 81, 82, 83
167ES	B44, 45, 82, 83
173K	Cw9, w10
177DT	(B7), B8, 60, 41, 42, 48, 81
184A	A2, 25, 26, 29, 32, 34, 43, 66, 68, 69, 74
184H	Cw1, w2, w9, w10, w4, w5, w6, w8, w12, w14, w15, w16, w18
193AV	A2, 25, 26, 29, 31, 32, 33, 34, 43, 66, 68, 69, 74
193PI	A1, 3, 9, 11, 30, 36, 80; B7, 8, 12, 13, 14, 16, 21, 22, 18, 27, 40, 41, 42, 46, 47, 48, 57, 59, 63, 69, 71, 72, 73, 75, 76, 77, 81, 82
211T	Cw2
219W	Cw1, w9, w10, w4, w14, w18
245AS	A25, 26, 29, 31, 32, 33, 34, 43, 66, 74
267PE	A80; Cw1, w2, w9, w10, w4, w5, w6, w8, w12, w14, w15, w16, w18

B50, B60 and B61), 76EDT (on B27, B37 and B47) and 167ES (on B44, B45, B82 and B83) (17, 25, 40, 49). We have also found human mAbs against 62QE paired with 56G and against 142MI paired with 79GT (40). Obviously, a complete determination of the class I epitope repertoire requires more studies on antibodies from sensitized patients. As illustrated above, the HLAMatchmaker-based analysis with single or paired eplets offers a useful approach.

Discussion

This report addresses the structure of HLA epitopes assigned by two different approaches. One is based on the reactivity patterns of antibodies with HLA-typed panels and the determination of amino acids within the antibody-binding span that is uniquely shared by antibody-reactive HLA antigens. Terasaki's group who have thus far identified 103 class I epitopes controlled by the HLA-A, -B and -C loci (4) has championed this method. The other approach applies a theoretical algorithm called HLA-Matchmaker that predicts epitopes based on polymorphic amino acid configurations within a 3 Å radius on the molecular surface and that are referred to as eplets.

Our analysis has shown that 93 (or 90%) of TerEps correspond to HLAMatchmaker-defined epitopes. Our eplet assignments required exactly the same antigen or group of antigens with a given TerEp. We considered also the amino acid descriptions of epitopes reported by Terasaki's group. The latter are solely based on sequence comparisons between antibody-reactive antigens and alleles, and often enough, a given epitope has two or more possible amino acid combinations, which makes its structural description

somewhat difficult to interpret. This empirical approach does not consider the immunizing event that led to the formation of specific antibody. Information about the HLA type of the antibody producer and the immunizing antigen permits a distinction between nonself and self amino acid residues shared between the immunizer and the antibody-reactive antigens in the Luminex panel. This information may also simplify the amino acid descriptions of TerEps. HLAMatchmaker incorporates the concept that each eplet has at least one nonself residue on the molecular surface. This matching algorithm applies two principles: (1) a mismatched HLA antigen may have an array of eplets as potential immunogens that can induce specific antibodies and (2) individuals cannot make antibodies against eplets that are expressed by their own HLA molecules including the intralocus or interlocus matches (22, 48).

Information about the immunogenic relationship between immunizing antigen and antibody producer is especially important for the understanding of epitopes defined by pairs of eplets separated far enough for contact by two different CDRs of antibody. Previous studies on human mAbs showed specificity patterns against a combination of nonself and self amino acid triplets that defined the epitopes (40). For instance, the reactivity of a 62QE-specific antibody required the presence of a glycine residue in position 56 found on the immunizing HLA-A3 molecule. Other 62QE-carrying alleles reacted with this mAb only if they had 56G. Similarly, the reactivity of two 142MI-specific antibodies required the presence of the GTLRG sequence in positions 79–83. These residues are located less than 15 Å from these triplets, and they appear to serve as a critical contact site for another CDR of antibody rather than binding to the specificity-determining CDR. Interestingly, 56G and 79GTLRG were self-residues present in the HLA antigens of the antibody producer. Other investigators have reported data that are consistent with the critical contact site concept (37–39). For instance, the Bw6-specific antibody SFR8-B6 recognizes an epitope defined by 75R, 79R and 82R (equivalent to the 79RN eplet), but its reactivity required also the presence of the 90A residue, which is about 10 Å away (50).

This analysis has shown that about one-third of TerEps correspond to eplet pairs. The eplets in such pairs could be both nonself or, alternatively, one is nonself and the other is self and serves as a critical contact site for antibody. While HLA typing information of the antibody producer would have provided definitive conclusions, we believe that most pairs consist of a nonself eplet that functions as the specificity site and a self-eplet that serves as a second critical contact site for antibody. The characterization of five TerEps equivalent to an eplet plus a locus-restricted residue supports this contention.

Table 3 shows 12 TerEps defined by eplets with permissible residue combinations. They reflect a structural cross-reactivity between eplets, whereby some residues play a dominant role and other residues have only a minor role in binding with antibody. Structural models suggest that these epitopes comprise short sequences of contiguous alignments of dominant and monomorphic residues as potential contact sites for specificity-determining CDRs of antibody.

Conversely, we identified TerEps that were dependent on antibody-inaccessible residues hidden below the molecular surface. Such residues were part of the eplet, but we identified three TerEp-defining eplets that required the presence of hidden residues 4–6 Å away. They are examples of epitopes whose conformation is dependent on hidden residues.

This analysis is not without some limitations. Besides the lack of HLA information for immunizer and antibody producer, the TerEp descriptions are based solely on amino acid comparisons between antibody-reactive and nonreactive antigens in the HLA panel used for antibody testing. More sophisticated methods such as the testing with informative HLA molecules mutated by site mutagenesis, and the crystallographic analyses of HLA antigen–antibody complexes will permit more detailed structural descriptions of HLA epitopes (38, 39, 51).

Nevertheless, this study shows how HLAMatchmaker can increase our understanding of HLA epitope immunogenicity (the ability to induce an antibody response) and antigenicity (the ability to react with antibody). HLAMatchmaker considers an eplet as a key element of an epitope, and specific aAbs can be induced only if the immunizing HLA antigen presents a nonself eplet that is absent from any antigen of the antibody producer. This analysis suggests that a mismatched eplet can induce specific antibodies with different reactivity patterns. An antibody may react with the following:

1. All antigens that carry a given eplet that can be expected to interact with its specificity-determining CDR loop.
2. Eplet-bearing antigens that share another amino acid configuration about 6–15 Å away and that would serve as a critical contact site for a second CDR loop. Such configuration could be a self-eplet, a nonself eplet or a locus-restricted amino acid sequence.
3. Only dominant residues in an eplet, whereas the other residues are permissible combinations that do not significantly alter epitope specificity. Such antibody will react with two or more eplets in the same sequence location, provided they share the same dominant residues. Moreover, an epitope may consist of an eplet with permissible residue combinations and that forms a pair with a second eplet or a locus-restricted sequence.
4. Eplets whose specific recognition is dependent on nearby hidden residues that alter eplet conformation.

These concepts are relevant to structural epitope antigenicity and the interpretation of antibody reactivity patterns with panels of HLA antigens. They will be incorporated in a new version of the HLAMatchmaker-based antibody analysis program, and this will add a new dimension to our understanding of eplet immunogenicity.

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References

1. Pei R, Lee JH, Shih NJ, Chen M, Terasaki PI. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. *Transplantation* 2003: **75**: 43–9.
2. El-Awar N, Cook DJ, Terasaki P. HLA class I epitopes: A and B loci. In: Terasaki P, Cecka M, eds. *Clinical Transplants 2006*, 79–94.
3. El-Awar N, Lee JH, Tarsitani C et al. HLA class I epitopes: recognition of binding sites by mAbs or eluted alloantibody confirmed with single recombinant antigens. *Human Immunol* 2007: **68**: 170–80.
4. El-Awar NR, Akaza T, Terasaki PI et al. Human leukocyte antigen class I epitopes: update to 103 total epitopes, including the C locus. *Transplantation* 2007: **84**: 532–40.
5. El-Awar N, Terasaki PI, Cai J et al. Epitopes of the HLA-A, B, C, DR, DQ and MICA antigens. *Clinical Transplants* 2007: 175–94.
6. Cai J, Terasaki PI, Mao Q et al. Development of nondonor-specific HLA-DR antibodies in allograft recipients is associated with shared epitopes with mismatched donor DR antigens. *Amer J Transplantation* 2006: **6**: 2947–54.
7. Deng CT, El-Awar N, Ozawa M et al. Human leukocyte antigen class II DQ alpha and beta epitopes identified from sera of kidney allograft recipients. *Transplantation* 2008: **86**: 452–9.
8. Cai J, Terasaki P. Post-transplantation antibody monitoring and HLA antibody epitope identification. *Curr Opin Immunol* 2008: **20**: 602–6.
9. Amit A, Mariuzza R, Phillips S, Poljak R. Three-dimensional structure of an antigen-antibody complex at 2.8 Angstrom resolution. *Science* 1986: **233**: 747–52.
10. Sheriff S, Silverton EW, Padlan EA et al. Three-dimensional structure of an antibody-antigen complex. *Proc Natl Acad Sci U S A* 1987: **84**: 8075–9.
11. Duquesnoy RJ. A structurally based approach to determine HLA compatibility at the humoral immune level. *Human Immunol* 2006: **67**: 847–62.
12. Davies D, Padlan E, Sheriff S. Antibody-antigen complexes. *Annu Rev Biochem* 1990: **59**: 439–73.
13. Braden B, Goldman E, Mariuzza R, Poljak R. Anatomy of an antibody molecule: structure, kinetics, thermodynamics and mutational studies of the antilysozyme antibody D. *Immunol Rev* 1998: **163**: 45–57.
14. Novotny J. Protein antigenicity: a thermodynamic approach. *Mol Immunol* 1991: **28**: 201–8.
15. Van Regenmortel M. Reductionism and the search for structure-function relationships in antibody molecules. *J Mol Recognit* 2002: **15**: 240–7.
16. DeLano W. Unraveling hot spots in binding interfaces: progress and challenges. *Curr Opin Struct Biol* 2002: **12**: 14–20.
17. Duquesnoy RJ, Marrari M. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. II. Verification of the algorithm and determination of the relative immunogenicity of amino acid triplet-defined epitopes. *Human Immunol* 2002: **63**: 353–63.
18. Lobashevsky AL, Senkbeil RW, Shoaf JL et al. The number of amino acid residues mismatches correlates with flow cytometry crossmatching results in high PRA renal patients. *Human Immunol* 2002: **63**: 364–74.
19. Duquesnoy RJ, Howe J, Takemoto S. HLAmatchmaker: a molecularly based algorithm for histocompatibility determination. IV. An alternative strategy to increase the number of compatible donors for highly sensitized patients. *Transplantation* 2003: **75**: 889–97.
20. Duquesnoy RJ, Takemoto S, De Lange P, Doxiadis IIN, Schreuder GMT, Claas FHJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination III. Effect of matching at the HLA-A, B amino acid triplet level on kidney transplant survival. *Transplantation* 2003: **75**: 884–9.
21. Boehringer D, Reinhard T, Duquesnoy R et al. Beneficial effect of matching at the HLA-A and B amino-acid triplet level on rejection free survival in penetrating keratoplasty. *Transplantation* 2004: **77**: 417–21.
22. Claas FHJ, Witvliet M, Duquesnoy RJ, Persijn G, Doxiadis IIN. The acceptable mismatch program as a fast tool to transplant highly sensitized patients awaiting a post-mortal kidney: short waiting time and excellent graft outcome. *Transplantation* 2004: **78**: 190–3.
23. Dankers MKA, Witvliet MD, Roelen DL et al. The number of amino acid triplet differences between patient and donor is predictive for the antibody reactivity against mismatched HLA antigens. *Transplantation* 2004: **128**: 1236–9.
24. Duquesnoy RJ, Witvliet MJ, Doxiadis IIN, de Fijter H, Claas FHJ. HLAMatchmaker-based strategy to identify acceptable HLA class I mismatches for highly sensitized kidney transplant candidates. *Transplant International* 2004: **7**: 31–8.
25. Adeyi OE, Girnita A, Awadalla Y et al. Serum analysis after kidney transplant nephrectomy reveals restricted antibody specificity patterns against donor HLA class I antigens. *Transpl Immunol* 2005: **14**: 53–62.
26. Claas FHJ, Dankers MK, Oudshoorn M et al. Differential immunogenicity of HLA mismatches in clinical transplantation. *Transplant Immunol* 2005: **14**: 187–91.
27. Doxiadis IIN, Duquesnoy RJ, Claas FHJ. Extending options for highly sensitized patients to receive a suitable kidney graft. *Curr Opin Immunol* 2005: **17**: 536–40.
28. Nambiar A, Duquesnoy RJ, Adams S et al. HLAMatchmaker-driven analysis of response to HLA

matched platelet transfusions in alloimmunized patients. *Blood* 2006: **107**: 1680–7.
29. Haririan A, Fagoaga O, Daneshvar H et al. Predictive value of HLA epitope matching using HLAMatchmaker for graft outcomes in a predominantly African-American renal transplant cohort. *Clin Transplant* 2006: **20**: 226–33.
30. Valentini RP, Nehlsen-Cannarella SL, Gruber SA et al. Intravenous immunoglubulin, HLA allele typing and HLAMatchmaker facilitate successful transplantation in highly sensitized pediatric renal allograft recipients. *Pediatr Transplant* 2007: **11**: 77–81.
31. Duquesnoy RJ. ''Match and Treat'', an effective strategy for transplanting highly sensitized pediatric transplant candidates? (Invited Editorial). *Pediatr Transplant* 2007: **11**: 3–4.
32. Duquesnoy R. Structural epitope matching for HLA alloimmunized thrombocytopenic patients: a new strategy to provide more effective platelet transfusion support? *Transfusion* 2008: **I48**: 221–7.
33. Duquesnoy R. Clinical usefulness of HLAMatchmaker in HLA epitope matching for organ transplantation. *Curr Opinion Immunol* 2008: **20**: 594–601.
34. Kosmoliaptsis V, Bradley J, Sharples L et al. Predicting the immunogenicity of human leukocyte antigen class I alloantigens using structural epitope analysis determined by HLAMatchmaker. *Transplantation* 2008: **85**: 1817–25.
35. Duquesnoy R. HLA class II antibodies and transplant outcome (invited analysis and commentary). *Transplantation* 2008: **86**: 638–40.
36. Duquesnoy R, Awadalla Y, Lomago J et al. Retransplant candidates have donor-specific antibodies that react with structurally defined HLA-DR,DQ,DP epitopes. *Transplant Immunol* 2008: **18**: 352–60.
37. Akkoc N, Scornik JC. HLA epitope matching. Contribution of matched residues to epitopes recognized by alloantibodies. *Transplantation* 1991: **52**: 903–7.
38. McCutcheon JA, Lutz CT. Mutagenesis around residue 176 on HLA-B*0702 characterizes multiple distinct epitopes for anti-HLA antibodies. *Human Immunol* 1992: **35**: 125–31.
39. Smith KD, Lutz CT. Alloreactive T cell recognition of MHC class I molecules. The T cell receptor interacts with limited regions of the MHC class I long alpha helices. *J Immunol* 1997: **158**: 2805–12.
40. Duquesnoy RJ, Mulder A, Askar M, Fernandez-Vina M, Claas FHJ. HLAMatchmaker-based analysis of human monoclonal antibody reactivity demonstrates the importance of an additional contact site for specific recognition of triplet-defined epitopes. *Hum Immunol* 2005: **66**: 749–61.
41. Duquesnoy R. Update on HLAMatchmaker: a molecularly based algorithm for histocompatibility determination at the epitope level. *EFI Newsletter* 2007: P 37–42 *and ASHI Quarterly* 2007: P 74–82.
42. Hogue C. Cn3D: a new generation of three-dimensional molecular structure viewer. *Trends Biochem Sci* 1997: **22**: 314–6.
43. Marsh SG, Bodmer JG. HLA-DR and -DQ epitopes and monoclonal antibody specificity. *Immunol Today* 1989: **10**: 305–12.
44. Klohe E, Fu XT, Ballas M, Karr RW. HLA-DR beta chain residues that are predicted to be located in the floor of the peptide-binding groove contribute to antibody-binding epitopes. *Human Immunol* 1993: **37**: 51–8.
45. Drover S, Marshall WH, Kwok WW, Nepom GT, Karr RW. Amino acids in the peptide-binding groove influence an antibody-defined, disease-associated HLA-DR epitope. *Scand J Immunol* 1994: **39**: 539–50.
46. Fu X-T, Drover S, Marshall WH, Karr RW. HLA-DR residues accessible under the peptide-binding groove contribute to polymorphic antibody epitopes. *Hum Immunol* 1995: **43**: 243–50.
47. Smith KD, Mace BE, Valenzuela A et al. Probing HLA-B7 conformational shifts induced by peptide-binding groove mutations and bound peptide with anti-HLA monoclonal antibodies. *J Immunol* 1996: **157**: 2470–8.
48. Duquesnoy RJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. *Human Immunol* 2002: **63**: 339–52.
49. Duquesnoy RJ, Claas FHJ. Progress Report of 14th International Histocompatibility Workshop Project on the structural basis of HLA Compatibility. *Tissue Antigens* 2007: **69** (Suppl. 1): 180–4.
50. Smith KD, Kurago ZB, Lutz CT. Conformational changes in MHC class I molecules. Antibody, T-cell receptor, and NK cell recognition in an HLA-B7 model system. *Immunologic Research* 1997: **16**: 243–59.
51. Hulsmeyer M, Chames P, Hillig R et al. A Major Histocompatibility Complex-Peptide-restricted antibody and T Cell Receptor molecules recognize their Target by distinct binding modes. Crystal structure of Human Leukocyte Antigen (HLA)-A1 -MAGE-A1 complex with Fab-Hyb3. *J Biol Chem* 2005: **280**: 2972–80.