TISSUE ANTIGENS
IMMUNE RESPONSE GENETICS

Tissue Antigens ISSN 0001-2815

Update of the HLA class I eplet database in the website based registry of antibody-defined HLA epitopes

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Key words
epitope; eplet; HLA-ABC; human leukocyte antigen

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Received 6 December 2013; revised 1 February 2014; accepted 5 February 2014

doi: 10.1111/tan.12322

Abstract

Eplets are small configurations of polymorphic amino acid residues on human leukocyte antigen (HLA) molecules and are considered as essential components of HLA epitopes recognized by antibodies. This report describes a new design of the eplet repertoire in HLA-ABC alleles used in Luminex kits for antibody testing. There were three steps (1): identify all combinations of polymorphic residues with HLA molecular modeling within a 3-Å radius, (2) determine polymorphic residue compositions of 3 Å patches from amino acid sequences of HLA alleles in Luminex panels and (3) annotate eplets from one or more patches present on one allele or shared by the same group of alleles. There are now 270 HLA-ABC eplets in the Registry, of which 219 are in antibody-accessible positions on the molecular surface and 51 are defined solely by residue polymorphisms located below the molecular surface. Each eplet has a list of Luminex and non-Luminex alleles for which mismatch acceptability can be determined.

Introduction

Antibody-defined human leukocyte antigen (HLA) epitopes can initiate humoral immune responses that lead to allograft rejection and transplant failure. Such epitopes include small configurations of polymorphic amino acid residues on the HLA molecular surface. Their structures are based on the concept that CDR-H3, one of the six complementarity determining region (CDR) loops of antibody, dominates the determination of antibody specificity (1–4). The initial structural model considered triplets, i.e. linear sequences of three amino acid residues (5), but this approach yielded an incomplete epitope repertoire. Eplets were introduced in 2006 as essential components of antibody-reactive HLA epitopes (6). They were defined by molecular modeling with residues within a 3-Å radius of antibody-accessible polymorphic residues on the molecular surface. Eplets considered both linear and discontinuous sequences and included also hidden residues not directly antibody-accessible. Since 2006, the HLAMatchmaker algorithm is based on eplets but various program modifications have led to changes in the repertoires and annotations of eplets. The recently established International Registry of Antibody-Defined HLA Epitopes (http://www.epregistry.com.br) (7) has also some inconsistencies with eplets in the HLAMatchmaker programs on the www.HLAMatchmaker.net website. Therefore, there was a need to go back to the drawing board so that a comprehensive and consistent eplet annotation system could be finalized for the HLA Epitope Registry.

Methods and results

The new eplet design consists of three general steps.

Step 1. Determination of sequence configurations for eplets

With Cn3D modeling (8) of HLA molecular structures, we can identify sequence positions of polymorphic residues within 3 Å of surface-exposed polymorphic residues. Table 1 represents a list of what has been estimated to be a complete set of sequence positions with up to three residue polymorphisms. Several positions are surrounded only by monomorphic residues and since eplet annotations use only polymorphic residues, the configurations have a single sequence number such as 1, 16 and 19. Other configurations are defined by two sequences (e.g. 14 + 17 and 45 + 46) or three sequences such as 44 + 45 + 46 and 62 + 63 + 65. Sequences 62–83 and 142–167 have many polymorphic positions in close proximity, most of which are well exposed, whereas others seem less antibody-accessible. Antibodies can be expected to recognize distinct configurations in overlapping sequences and multiple possibilities have been considered. For instance, there are seven configurations for

position 62: one with the hidden 63, two with the exposed 65 or 66, three combined with 63, 65 and/or 66 and finally 62 alone. The hidden 63 position is also very close to 65 and 66 and there are three possible configurations involving this position.

Altogether, Table 1 lists 116 configurations for exposed positions. It should be noted that they include continuous and discontinuous sequences.

The new eplet design addressed also other polymorphic residues in hidden positions that appear antibody-inaccessible. Many of them are in the peptide-binding groove. Examples are positions 9, 11 and 12. It is possible that they affect the surface expression of nearby monomorphic residues thereby creating epitopes recognized by antibodies. Table 1 includes 21 configurations solely defined by hidden polymorphic residues. Such epitopes might have very minimal immunogenicity, but they are included in the Registry.

Step 2. Generation of patches defined by residue polymorphisms and selection of annotated eplets

The www.HLAMatchmaker.net website has an Excel program called 'HLAPatch Generator' developed by Dr Grzegorz Dudek (Czestchowa University of Technology, Częstochowa, Poland). After entering the sequence positions of each configuration, the program generates a repertoire of HLA alleles with patches defined by residue compositions of each configuration recorded in the data entry. The program can also generate a list of patch-carrying HLA alleles.

The initial analysis was limited to alleles commonly used in commercially available Luminex assays with single alleles; serum reactivity analysis would detect only antibodies specific for the epitopes expressed on such panels. Using the HLA alleles in current Luminex panels for antibody detection, HLAPatch Generator has generated 620 patches from 116

Table 1 Configurations of polymorphic sequence positions on class I human leukocyte antigen (HLA) molecules

Exposed	Res	Res	Res	Exposed	Res	Res	Res	Exposed	Res	Res	Res	Hidden	Res	Res
1	1			73	73	74	76	149	149	151		4	4	
14	14	17		73	73	76		150	150	151	152	6	6	
16	16			73	73	76	77	150	150	151		9	9	
19	19			73	73			150	150			11	11	12
41	41			76	76	77		151	151			21	21	
44	44	45	46	76	76	77	79	151	151	152		24	24	
45	45	46		76	76	79		156	156			30	30	
56	56			76	76	77	80	156	156	158		32	32	
62	62	63		76	76	80		158	158			35	35	
62	62	63	65	76	76	79	80	161	161			49	49	
62	62	63	66	76	76			162	162	163	167	67	67	
62	62	65		77	77	79	80	163	163			70	70	
62	62	65	66	80	80	81	82	163	163	166		74	74	
62	62	66		80	80			163	163	166	167	94	94	95
62	62			80	80	81		166	166	167		97	97	
63	63	65		80	80	82	83	166	166			99	99	
63	63	66		82	81	82	83	170	170	171		113	113	114
63	63	65	66	82	82	83		173	173			116	116	
65	65	66		82	82			177	177			152	152	
65	65	66	67	90	90	91		177	177	178		156	156	
65	65	66	69	102	102	103		180	180			199	199	
65	65	69		105	105			182	182	183	184			
65	65			107	107			186	186					
66	66	67		109	109			193	193	194				
66	66	67	69	127	127			193	193					
66	66	69		131	131			194	194					
66	66	69	70	138	138			207	207					
66	66			138	138	142		211	211					
69	69	70	71	142	142	143	144	219	219					
69	69	70		142	142	145		245	245	246				
69	69	70	73	144	144	145		247	247					
69	69	71		144	144	149		248	248					
69	69	73		144	144			249	249					
69	69	73	77	145	145	149		253	253					
69	69			145	145			261	261	270				
70	70	73		147	147			270	270	271				
70	70			149	149	150	151	273	273					
71	71	73	77	149	149	150		275	275	276				
73	73	74		149	149									

exposed and 21 hidden residues. These patches are described by sequence numbers and standard single letter amino acid residue notations. They were sorted according to the Luminex alleles encoded by HLA-A ($N_{max} = 35$), followed by HLA-B ($N_{max} = 59$) and finally HLA-C ($N_{max} = 23$). A detailed list of these patches is on the www.HLAMatchmaker.net website and a few rules have been applied to select annotated eplets. First, a patch was considered monomorphic if it was present on N_{max} alleles of any locus; 80 such patches were ruled out as potential epitopes because being self they cannot be considered immunogenic.

Many of the remaining 530 patches are shared by identical groups of Luminex alleles; the second rule was to select one patch from a given group of patches as the annotated eplet. As an example, nine patches (73TAN, 73TA, 76ANG, 76AGT, 76AG, 76AT, 76A, 76AN and 76ANT) are shared by the same seven alleles *A*01:01*, *A*26:01*, *A*29:01*, *A*29:02*, *A*36:01*, *A*43:01* and *A*80:01*. It is possible that these alleles share overlapping epitopes representing 76A as a key residue and surrounded by different combinations of 73T, 77N, 79G and/or 80T. Current Luminex panels cannot resolve this issue, but it seems impractical to include each patch as a potential epitope. Therefore, the 76ANT patch was chosen as the annotated eplet and it is defined by residues in the 73T + 76A + 77N + 79G + 80T combination.

Certain alleles share multiple configurations that correspond to two or more eplets in different sequence positions but which cannot be distinguished from each other because there are no informative alleles in the Luminex panel. For instance, *A*01:01* and *A*36:01* share 44KM, 149AVH, 149AV, 150V, 150VH, 150VHA, 156RV and 158V. This collection of patches could comprise different epitopes in three separate configurations on the molecular surface, namely 44K + 45M, 149A + 150V + 151H + 152A and 156R + 158V. In this case, the eplet annotation $44KM_3$ is used whereby the subscript represents three separate configurations.

There are also overlapping eplets that are shared between similar but not identical groups of Luminex alleles. As an example, six eplets, 62RR, 62RNR, 62RN, 63NN, 62RRN and 62RTN, are on *A*25:01*, *A*26:01*, *A*33:01/03*, *A*34:01/02*, *A*66:01/02*, *A*68:01/02* and *A*69:01* (Table 2). These eplets have 62R in combination with 63N (which is less well exposed), 65R and/or 66N. The 62RR (62R + 65R) and 62RNR (62R + 63N + 65R) eplets are on all of the above HLA-A alleles; *B*15:16* that has 63E rather than 63N is on 62RR but not on 62RNR. It should be noted that 62RN (62R + 63N) is also on 32 HLA-B alleles including B7, B8 and B14. The 63NN, 62RRN and 62RTN eplets are on all 62R-carrying HLA-A alleles except *A*34:01* that has 66K rather than 66N, and there are some additional differences: 62RRN is on *B*15:16*, whereas 62RTN is on *B*15:16*, *C*07:01* and *C*15:02*.

The differences between these eplets with such close structural relationships might be useful in interpreting HLA antibody specificity patterns. Suppose, a patient produces a specific antibody to a 62R-related epitope induced by an immunizing allele such as *A*25:01*. From the reactivity patterns with the alleles shown in Table 2, one can determine which eplets are recognized by specific antibodies. Such information could explain unexpected antibody reactivity with alleles like *B*15:16*, *C*07:01* and *C*15:02* after sensitization by *A*25:01*.

Step 3. Finalization of the HLA-ABC eplet repertoire

A total of 270 HLA-ABC eplets have been annotated from 620 patches and recorded in the HLA Epitope Registry; all of them are on HLA alleles in Luminex panels. There are 219 eplets that have one or more polymorphic residues in antibody-accessible locations on the molecular surface including 117 eplets in the α1 domain, 70 eplets in the α2 domain and 24 eplets in the α3 domain. Table 3 shows that eplets are present on one or more alleles of a single locus or on alleles encoded by two or three class I loci. Several eplets such as 1C, 44RME and 66I are shared by a majority of alleles encoded by a given locus, whereas others such as 73TD, 142ITQ and 156LA are shared between multiple alleles encoded by two or three loci. Such high-frequency eplets are not often mismatched but specifically induced immune responses may lead to highly reactive antibodies with allele panels. The http://www.epregistry.com.br website has detailed information for each eplet such as residue descriptions, eplet frequencies and lists of eplet-carrying alleles including those on Luminex panels.

Table 2 Comparisons of 62R-related eplets on Luminex alleles

Eplet	Residues	*A*25:01*	*A*26:01*	*A*33:01*	*A*33:03*	*A*34:01*	*A*34:02*	*A*66:01*	*A*66:02*	*A*68:01*	*A*68:02*	*A*69:01*	*B*15:16*	Other
62RR	62R65R	+	+	+	+	+	+	+	+	+	+	+	+	
62RNR	62R63N65R	+	+	+	+	+	+	+	+	+	+	+	Has 63E	
62RN	62R63N	+	+	+	+	+	+	+	+	+	+	+	Has 63E	32 HLA-B alleles[a]
63NN	62R63N65R66N	+	+	+	+	Has 66K	+	+	+	+	+	+	Has 63E	
62RRN	62R65R66N	+	+	+	+	Has 66K	+	+	+	+	+	+	+	
62RTN	62R64T66N[b]	+	+	+	+	Has 66K	+	+	+	+	+	+	+	*C*07:01, C*15:02*

[a]On 32 HLA-B alleles of B7, B8, B14, B16, B18, B22, B35, B42, B51, B59, B62, B63, B70, B73, B78, B81 and B82

[b]The annotation includes the monomorphic 64T to distinguish this eplet from 62RN

Table 3 Eplets with residue polymorphisms in antibody-accessible positions on the HLA molecular surface

Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total
1C	0	0	17	17	71ATN	0	4	4	8	145KHA	7	0	0	7
16S	0	0	3	3	71ATS	0	11	8	19	145RT	7	0	0	7
17RS	2	0	0	2	71HS	22	0	0	22	147L	0	3	4	7
17WR	0	0	1	1	71KA	0	4	0	4	149AH	14	0	0	14
19K	1	0	0	1	71QS	13	0	0	13	149TAH	8	0	0	8
41T	0	13	0	13	71SA	0	4	0	4	150AAH	12	0	0	12
43RRM	2	0	0	2	71STN	12	0	0	12	150AH	20	0	0	20
$44KM_3$	2	0	0	2	71TTD	0	2	0	2	151AHA	2	0	0	2
44RM	33	12	0	45	71TTN	0	12	0	12	151AHE	9	0	0	9
44RMA	0	11	0	11	71TTS	0	27	0	27	151AHV	9	0	0	9
44RME	33	0	0	33	72QTD	25	22	12	59	151ARV	9	35	0	44
44RT	0	10	0	10	73ID	3	0	0	3	151H	22	0	0	22
45EE	0	26	0	26	73AN	0	0	6	6	152HA	4	0	0	4
45KE	0	11	0	11	73AS	0	0	5	5	152RA	0	0	4	4
$56E_4$	1	0	0	1	73TD	17	4	0	21	152RE	0	24	18	42
56R	3	0	0	3	73TDA	6	0	0	6	152RR	2	0	0	2
62EE	5	0	0	5	73TDE	7	20	0	27	152RT	0	0	1	1
62GE	5	3	0	8	73TDV	12	2	12	26	152RW	1	0	0	1
$62GK_2$	5	0	0	5	73TN	12	16	4	32	156DA	0	8	1	9
62GRN	0	3	0	3	73TS	2	37	9	48	156LA	16	40	8	64
62LQ	3	0	0	3	73TV	17	2	12	31	156QA	4	0	1	5
62QE	10	0	0	10	73TVD	17	0	0	17	156RA	0	3	8	11
62REN	0	1	2	3	73TVN	0	0	4	4	156WA	12	4	5	21
62RER	0	1	0	1	73TVS	0	1	8	9	158T	0	4	0	4
62RK	1	1	21	23	73TY	0	37	0	37	161D	1	0	0	1
62RN	11	32	0	43	76ANT	7	0	0	7	162DLS	0	1	0	1
62RNQ	0	32	0	32	76ED	0	4	0	4	162GLS	0	4	0	4
62RNR	11	0	0	11	76EG	1	0	0	1	163E	2	14	3	19
62RR	11	1	0	12	76EN	5	16	0	21	163EW	1	14	3	18
62RRN	10	1	0	11	76ENI	4	12	0	16	163L	0	29	3	32
62RTN	10	1	2	13	76ENR	4	16	0	20	163LE	0	28	3	31
63EI	0	22	0	22	76ENT	1	4	0	5	163LG	0	1	0	1
63EK	9	1	21	31	76ES	2	37	0	39	163LW	0	23	3	26
63EN	11	4	2	17	76ESI	2	0	0	2	163R	7	0	0	7
63ER	16	4	0	20	76ESN	0	37	0	37	163RG	1	0	0	1
63ERN	12	4	0	16	76ET	1	8	0	9	163RW	6	0	0	6
63NN	10	0	0	10	76VDT	20	0	0	20	163T	25	16	17	58
65GK	4	0	0	4	76VRN	0	2	13	15	163TEW	22	16	17	55
65QIA	0	13	0	13	76VS	0	1	13	14	163TG	3	0	0	3
65QKR	0	1	21	22	77NGT	8	0	0	8	166DG	5	1	0	6
65QNR	0	0	2	2	77SRN	0	38	13	51	166ES	0	5	0	5
65RA	30	4	0	34	79GT	28	0	0	28	170RH	1	9	0	10
65RK	6	0	0	6	80I	6	12	0	18	170RY	35	50	16	99
65RNA	24	4	0	28	80K	0	0	10	10	173K	0	0	3	3
66I	0	54	0	54	80N	0	39	13	52	177DK	0	5	0	5
66IC	0	13	0	13	80T	28	8	0	36	177DT	0	3	0	3
66ICT	0	9	0	9	80TA	0	4	0	4	177KT	0	0	4	4
66IF	0	8	0	8	80TL	28	4	0	32	180E	0	8	0	8
66IS	0	22	0	22	80TLR	0	8	0	8	182TDP	15	0	0	15
66IY	0	11	0	11	81ALR	6	16	0	22	184A	19	0	0	19
66IYT	0	2	0	2	82LR	6	20	0	26	184H	0	0	19	19
66K	10	1	21	32	90AR	0	0	1	1	$184R_3$	0	0	1	1
66KA	10	0	0	10	90D	11	1	8	20	186R	1	0	0	1
66KAH	9	0	0	9	102DV	33	42	3	78	193AV	23	0	0	23
66N	24	4	2	30	102HV	1	0	0	1	193LV	0	0	1	1
66NAH	12	0	0	12	103L	0	16	20	36	193PI	12	51	0	63
66NAQ	12	0	0	12	$103M_2$	0	1	0	1	$193PL_3$	0	0	3	3
66NM	2	4	0	6	105S	21	0	0	21	193PV	0	8	19	27
66NV	23	0	0	23	107W	6	0	0	6	194V	22	8	20	50

Table 3 Continued

Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total
66RKQ	1	0	0	1	109F	32	0	0	32	207S	23	0	0	23
69AA	0	17	0	17	109FE	31	0	0	31	211T	0	0	1	1
69AQT	13	9	0	22	127K	12	0	0	12	219W	0	0	9	9
69AT	31	17	0	48	131S	0	47	0	47	245AS	14	0	0	14
69ATD	17	2	0	19	138K	0	0	2	2	245TA	0	2	0	2
69ATN	12	4	0	16	138MI	26	0	0	26	245VA	2	0	0	2
69ATS	2	10	0	12	142ITQ	18	56	22	96	248M	0	0	1	1
69RA	0	0	11	11	143S	0	3	1	4	253Q	22	1	4	27
69RT	0	1	12	13	144K	16	0	0	16	267PE	1	0	19	20
69TNT	0	41	0	41	144KA	15	0	0	15	267QE	0	1	4	5
70HT	18	0	0	18	144QL	0	2	0	2	270C	0	1	1	2
70IAQ	0	9	0	9	144TKH	8	0	0	8	275EL	7	0	0	7
70QT	13	10	12	35	144KR	8	0	0	8	275G	0	0	3	3
71ATD	0	2	0	2	145HT	1	0	0	1	275K	0	1	5	6

The repertoire has also 51 eplets that are solely defined by residue polymorphisms in antibody-inaccessible positions, most of them are in the peptide-binding groove (Table 4). Such residues may influence the conformation of nearby monomorphic residues on the molecular surface, thereby giving rise to epitopes that might be recognized by specific antibodies. The question remains, however, whether any such epitope is actually immunogenic.

The eplet repertoire has been generated with alleles used in commercial Luminex kits for HLA antibody analysis. What is the eplet expression on other non-Luminex alleles? There are ready answers for eplets that have been annotated from a single patch. For instance, 62GE is shared by the Luminex alleles *A*02:01/02/03/05/06*, *B*57:01/03* and *B*58:01*. Non-Luminex alleles such as *A*02:04*, *B*57:02*, *B*58:02* and even *A*24:08* have 62GE, but others such as *A*02:55* (62RN) and *B*58:04* (62EE) have a different eplet. In other words, it is quite easy to determine mismatch acceptability for 62GE. The situation may change if an annotated eplet has been selected from multiple patches shared by the same allele(s) as illustrated in four examples.

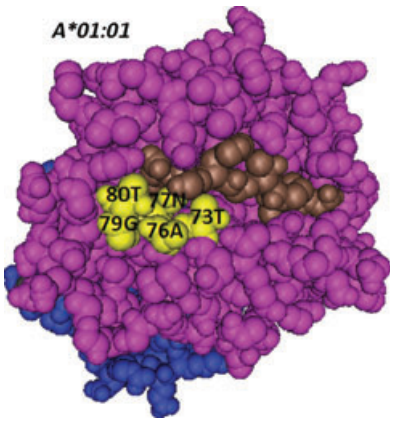
Example 1: As described above, the 76ANT eplet has been selected from nine patches (73TAN, 73TA, 76ANG, 76AGT, 76AG, 76AT, 76A, 76AN and 76ANT) that are shared by the same Luminex alleles *A*01:01*, *A*26:01*, *A*29:01*, *A*29:02*, *A*36:01*, *A*43:01* and *A*80:01*. This eplet is represented by 76A as a key residue, which is surrounded by 73T, 77N, 79G and 80T. It is possible that different residue combinations with 76A may lead to two or more distinct epitopes which cannot be distinguished by antibodies tested with current Luminex panels. Table 5 shows that most non-Luminex alleles in the *A*01*, *A*26*, *A*29* and *A*36* group have the

Table 4 Eplets based solely on residue polymorphisms in antibody-inaccessible sequence positions

Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total
9D	0	1	6	7	94IL	0	0	1	1	113HD	0	16	1	17
9F	10	0	1	11	94TF	0	0	1	1	113HN	0	26	0	26
9H	0	14	0	14	94TI	25	1	1	27	113YD	0	4	14	18
9S	6	0	2	8	94TL	6	31	17	54	113YH	11	4	0	15
9T	5	0	0	5	94TV	4	0	0	4	113YN	0	9	8	17
9Y	14	44	14	72	94TW	0	16	0	16	113YQ	12	0	0	12
11AM	0	37	0	37	97I	9	0	0	9	113YR	10	0	0	10
11AV	0	3	20	23	97M	13	0	0	13	116D	22	6	0	28
11SM	1	0	0	1	97N	0	3	0	3	116F	0	10	9	19
11SV	34	19	3	56	97R	13	32	18	63	116L	0	11	1	12
21H	0	0	7	7	97S	0	9	0	9	116S	0	15	10	25
24S	0	21	7	28	97T	0	11	0	11	116Y	11	17	3	31
24T	0	17	0	17	97V	0	2	0	2	152A	4	0	4	8
30G	0	1	0	1	97W	0	2	5	7	152E	9	24	18	51
32L	0	14	0	14	99F	4	3	5	12	152V	19	35	0	54
35Q	1	0	3	4	99S	0	1	1	2	156L	17	44	8	69
94II	0	11	3	14	99Y	31	55	13	99	156R	2	3	8	13
										199V	0	2	0	2

Table 5 Comparisons of relevant amino acid configurations between 76ANT-carrying Luminex alleles and non-Luminex alleles

Selected alleles		73	76	77	79	80	Selected alleles	73	76	77	79	80	Selected alleles		73	76	77	79	80
*A*01:01*	LUM	T	A	N	G	T	*A*26:06*	T	V	D	G	T	*A*26:37*		T	A	N	G	T
*A*01:02*		T	A	N	G	T	*A*26:07*	T	A	N	G	T	*A*29:01*	LUM	T	A	N	G	T
*A*01:03*		T	A	N	G	T	*A*26:08*	T	A	N	G	T	*A*29:02*	LUM	T	A	N	G	T
*A*01:06*		T	A	N	G	T	*A*26:09*	T	A	N	G	T	*A*29:03*		T	A	N	G	T
*A*01:07*		T	E	N	G	T	*A*26:10*	T	A	N	G	T	*A*29:04*		T	A	N	G	T
*A*01:08*		T	A	N	G	T	*A*26:12*	T	A	N	G	T	*A*29:05*		T	A	N	G	T
*A*01:09*		T	A	N	G	T	*A*26:13*	T	A	N	G	T	*A*29:06*		T	A	N	G	T
*A*01:10*		T	A	N	G	T	*A*26:14*	T	A	N	G	T	*A*29:07*		T	A	N	G	T
*A*01:12*		T	A	N	G	T	*A*26:15*	T	A	N	G	T	*A*29:09*		T	A	N	G	T
*A*01:13*		T	V	D	G	T	*A*26:16*	T	A	N	G	T	*A*29:10*		T	A	N	G	T
*A*01:14*		T	A	N	G	T	*A*26:17*	T	A	N	G	T	*A*29:11*		T	A	N	G	T
*A*01:17*		T	A	N	G	T	*A*26:18*	T	A	N	G	T	*A*29:12*		T	A	N	G	T
*A*01:19*		T	A	N	G	T	*A*26:19*	T	A	N	G	T	*A*29:13*		T	E	S	R	I
*A*01:20*		T	A	N	G	T	*A*26:20*	T	A	N	G	T	*A*29:14*		T	A	N	G	T
*A*01:21*		T	A	N	G	T	*A*26:21*	T	V	D	G	T	*A*29:15*		T	A	N	G	T
*A*01:23*		T	A	N	G	T	*A*26:22*	T	A	N	G	T	*A*29:16*		T	A	N	G	T
*A*01:24*		T	A	N	G	T	*A*26:23*	T	A	N	G	T	*A*29:17*		T	A	N	G	T
*A*01:25*		T	A	N	G	T	*A*26:24*	T	A	N	G	T	*A*29:18*		T	A	N	G	T
*A*01:26*		T	A	N	G	T	*A*26:26*	T	A	N	G	T	*A*29:19*		T	V	D	G	T
*A*01:28*		T	V	D	G	T	*A*26:27*	T	A	N	G	T	*A*36:01*	LUM	T	A	N	G	T
*A*01:29*		T	A	N	G	T	*A*26:28*	T	A	N	G	T	*A*36:02*		T	A	N	G	T
*A*01:30*		T	A	N	G	T	*A*26:29*	T	A	N	G	T	*A*36:03*		T	A	N	G	T
*A*01:32*		T	A	N	G	T	*A*26:30*	T	V	D	G	T	*A*36:04*		T	A	N	G	T
*A*01:33*		T	A	N	G	T	*A*26:31*	T	A	N	G	T	*A*43:01*	LUM	T	A	N	G	T
*A*26:01*	LUM	T	A	N	G	T	*A*26:32*	T	A	N	G	T	*A*80:01*	LUM	T	A	N	G	T
*A*26:02*		T	A	N	G	T	*A*26:33*	I	A	N	G	T	*A*11:17*		T	A	N	G	T
*A*26:03*		T	V	D	G	T	*A*26:34*	T	A	N	G	T	*A*11:40*		T	A	N	G	T
*A*26:04*		T	A	N	G	T	*A*26:35*	T	A	N	G	T	*A*24:04*		T	A	N	G	T
*A*26:05*		T	E	N	G	T	*A*26:36*	T	A	N	G	T	*A*74:10*		T	A	N	G	T



Residue locations for the 76ANT eplet

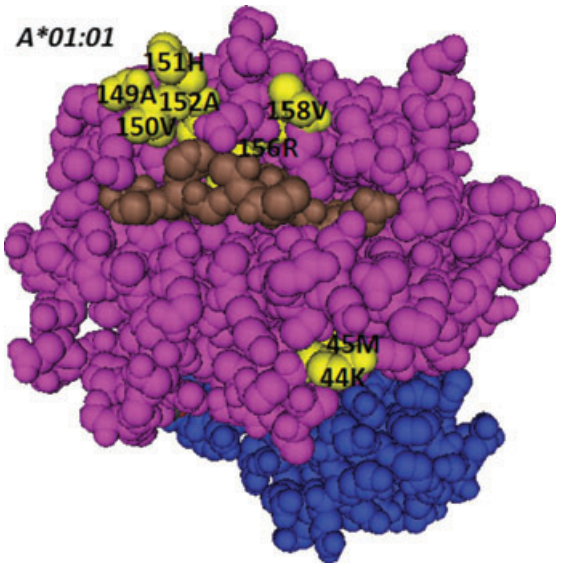
73T + 76A + 77N + 79G + 80T combination, and therefore, the 76ANT eplet annotation would be correct for them. The molecular model shows the locations of these residues on *A*01:01*. Four unrelated alleles *A*11:17*, *A*11:40*, *A*24:04* and *A*74:10* have also 76ANT. On the other hand, 76ANT is not on *A*01:07* and *A*26:05*, which have 76E rather than 76A, as well as on *A*01:13*, *A*01:28*, *A*26:03*, *A*26:06*, *A*26:21*, *A*26:30* and *A*29:19*, which have 76V and 77D. These alleles would be acceptable mismatches for cases with 76ANT antibodies. No alleles that carry 76A but have differences for 73T, 77N, 79G or 80T have been found. Altogether, a determination of mismatch acceptability for 76ANT can be readily determined for this group of non-Luminex *A*01*, *A*26*, *A*29* and *A*36* alleles.

Example 2: As described above, the $44KM_3$ eplet may reflect three epitopes described by configurations in separate locations on the molecular surface, namely 44K + 45M, 149A + 150V + 151H + 152A and 156R + 158V. This eplet is unique for *A*01:01* and *A*36:01*. Table 6 shows that 17 of 24 *A*01* and 2 of 3 *A*36* non-Luminex alleles have exactly the same residue configurations and they are referred to as $44KM_3$-carrying. For these alleles, it does not matter which epitope configuration is recognized by patient's antibodies; they are all unacceptable mismatches. In contrast, the remaining *A*01* and *A*36* alleles have 44K + 45M, but there are differences in the 149–152 and 156–158 sequence locations. Five *A*01* alleles: *A*01:10*, *A*01:12*, *A*01:19*, *A*01:21* and *A*01:26* have different residues in positions 149–152; they would not react with 149A + 150V + 151H + 152A-specific antibodies. Moreover, *A*01:06*, *A*01:12*, *A*01:19*, *A*01:25* and *A*36:02* have different residues in the 156–158 sequence, and they can be expected to be non-reactive with 156R + 158V-specific antibodies. Thus, a determination of mismatch acceptability of *A*01* and *A*36* alleles depends on what epitope is specifically recognized by *A*01:01* + *A*36:01*-reactive antibodies. Table 6 implies that antibody specificity for 44KM will render all *A*01* and *A*36* alleles as unacceptable mismatches. Antibody specificity for 149AVH rather than 44KM will determine some *A*01* alleles as acceptable mismatches, whereas other alleles such as *A*03:18* and *A*68:41* are unacceptable mismatches because they have 149AVH.

Example 3: Table 2 compares the presence of six overlapping 62R-containing eplets on the HLA-A alleles *A*25:01*, *A*26:01*, *A*33:01/03*, *A*34:01/02*, *A*66:01/02*, *A*68:01/02* and *A*69:01* in the Luminex panel. From the reactivity patterns, with these antigens as well as *B*15:16*, *C*07:01* and *C*15:02*, one can determine which eplet is recognized by a specific antibody. This information is relevant to the determination of mismatch acceptability of non-Luminex alleles. Table 7 has examples on how such alleles might be predicted as acceptable or unacceptable mismatches in relation to antibody specificities towards five eplets: 62RR, 62RNR, 63NN, 62RRN and 62RTN. *A*34:04* and *A*68:12* that have 62R + 63N + 65R + 66N would be considered as unacceptable mismatches regardless of which one of these eplets is recognized. *A*34:05* has 62R + 63N + 65R + 66K and would be unacceptable for cases with antibodies specific for 62RR and/or 62RNR but acceptable for cases with antibodies against 63NN, 62RRN and/or 62RTN. Similarly, *A*68:13*, *B*15:17* and *B*15:67* that have 62R + 63E + 65R + 66N would be unacceptable for cases with antibodies to 62RR, 63RRN and/or 62RTN. The same principle can be applied to determine mismatch acceptability for three HLA-C alleles and two *HLA-A*02* alleles with informative residue configurations (Table 7). These findings indicate that the usefulness of detailed information about eplet specificities of antibodies can identify non-Luminex alleles as acceptable mismatches.

Table 6 Residue configurations of non-Luminex alleles in relation to an eplet annotated as $44KM_3$ and shared between *A*01:01* and *A*36:01*

Alleles		44	45	149	150	151	152	156	158
A*01:01 **LUM**	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*36:01 **LUM**	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:02	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:03	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:06		**K**	**M**	**A**	**V**	**H**	**A**	**L**	**A**
A*01:07	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:08	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:09	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:10		**K**	**M**	**A**	**A**	**R**	**R**	**R**	**V**
A*01:12		**K**	**M**	**A**	**A**	**H**	**V**	**Q**	**A**
A*01:13	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:14	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:17	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:19		**K**	**M**	**A**	**A**	**H**	**V**	**Q**	**A**
A*01:20	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:21		**K**	**M**	**A**	**A**	**H**	**V**	**R**	**V**
A*01:23	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:24	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:25		**K**	**M**	**A**	**V**	**H**	**A**	**Q**	**V**
A*01:26		**K**	**M**	**A**	**A**	**H**	**A**	**R**	**V**
A*01:28	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:29	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:30	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:32	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:33	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*01:35	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*36:02		**K**	**M**	**A**	**V**	**H**	**A**	**R**	**A**
A*36:03	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*36:04	**$44KM_3$**	**K**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*03:18		**R**	**M**	**A**	**V**	**H**	**A**	**R**	**V**
A*68:41		**R**	**M**	**A**	**V**	**H**	**V**	**W**	**A**



Locations of three amino acid configurations corresponding to the $44KM_3$ eplet

Example 4: The Luminex alleles *B*27:03*, *B*27:05*, *B*37:01* and *B*47:01* share a unique antibody-reactive epitope that corresponds to the 76ED and/or 80TLL eplets. The 76E and 82L residues are 7 Å from each other (see figure in Table 8), and with the current Luminex panel we cannot have an answer to the question whether specific antibodies react primarily with 76DE or 80TLL or both. This question seems especially relevant to the determination of mismatch acceptability of non-Luminex alleles, especially those in the B27 group would react with 76ED- and/or 80TLL-specific antibodies. Table 8 summarizes the residue information in relevant sequence positions: 76E + 77D + 80T + 81L + 82L. Large groups of B27 and B37 alleles (*B*27:07/09/10*, etc.; *B*37:02/04/06*, etc., respectively) as well as *B*47:05* and four unrelated alleles (*B*07:27*, *B*15:43*, *B*38:17* and *B*53:03*) have the same sequence configuration as the Luminex alleles. These alleles would be unacceptable mismatches for patients with antibodies reacting with the *B*27:03*, *B*27:05*, *B*37:01* and *B*47:01* group and a distinction between 76ED- and 80TLL-specific crecognition is unnecessary.

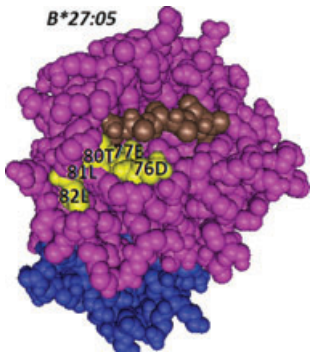
On the other hand, *B*27:42* and *B*37:05* have 76ED but 81NLR instead of 80TLL. These alleles will react with 76ED-specific antibodies but not with 81TLL-specific antibodies. Eleven B27 alleles (*B*27:04/06/11*, etc.) have 80TLL but not 76ED. These alleles will react with 80TLL-specific antibodies

Table 7 Examples of mismatch acceptability of non-Luminex alleles in the presence of antibodies to 62R-related eplets

Non-luminex allele 62, 63, 65, 66 Residues Antibody specific for	*A*34:04* RNRN	*A*34:05* RNRK	*A*68:12* RNRN	*A*68:13* RERN	*B*15:17* RERN	*B*15:67* RERN	*C*07:03* REQK	*C*07:06* REQN	*C*15:05* REQN	*A*02:55* RNRN	*A*02:143* RERN
62RR	unacc	unacc	unacc	unacc	unacc	unacc	acc	acc	acc	unacc	unacc
62RNR	unacc	unacc	unacc	acc	acc	acc	acc	acc	acc	unacc	acc
63NN	unacc	acc	unacc	acc	acc	acc	acc	acc	acc	unacc	acc
62RRN	unacc	acc	unacc	unacc	unacc	unacc	acc	acc	acc	unacc	unacc
62RTN	unacc	acc	unacc	unacc	unacc	unacc	acc	unacc	unacc	unacc	unacc

Table 8 Residue configurations of non-Luminex alleles in relation to the 76ED and 80TLL eplets shared between Luminex alleles *B*27:03*, *B*27:05*, *B*37:01* and *B*47:01*

Alleles	76ED/80TLL Eplet	76	77	80	81	82
B*27:03 LUM	**76ED and/or 80TLL**	**E**	**D**	**T**	**L**	**L**
B*27:05 LUM	**76ED and/or 80TLL**	**E**	**D**	**T**	**L**	**L**
B*37:01 LUM	**76ED and/or 80TLL**	**E**	**D**	**T**	**L**	**L**
B*47:01 LUM	**76ED and/or 80TLL**	**E**	**D**	**T**	**L**	**L**
B*27:07/09/10/13/14/16/17/19/27/28/	**76ED and/or 80TLL**	**E**	**D**	**T**	**L**	**L**
29/32/34/35/37/38/39/41/43/45/46/50	**76ED and/or 80TLL**	**E**	**D**	**T**	**L**	**L**
B*37:02/04/06/07/08/09/12/13, B*47:05	**76ED and/or 80TLL**	**E**	**D**	**T**	**L**	**L**
B*07:27, B*15:43, B*38:17, B*53:03	**76ED and/or 80TLL**	**E**	**D**	**T**	**L**	**L**
B*27:42, B*37:05	**76ED only**	**E**	**D**	**N**	**L**	**R**
B*27:04/06/11/15/20/21/23/24/25/31/36	**80TLL only**	**E**	**S**	**T**	**L**	**L**
B*27:08 LUM	**not76ED/80TLL**	**E**	**S**	**N**	**L**	**R**
B*27:12/18/26/33/40/44, B*37:11, B*47:02	**not76ED/80TLL**	**E**	**S**	**N**	**L**	**R**
B*37:34, B*47:03	**not76ED/80TLL**	**E**	**S**	**N**	**L**	**L**
B*27:01/02/30, B*37:10, B*47:04	**not76ED/80TLL**	**E**	**N**	**T**	**A**	**L**



Locations of 76ED and 80TLL on the molecular surface. The distance between 76D and 82L is 7 Ångstroms.

but not with 76ED-specific antibodies. Since current Luminex panels cannot distinguish whether a patient has 76ED-specific or 80TLL-specific antibodies, it is difficult to ascertain the mismatch acceptability of these alleles.

It should be noted that the Luminex allele *B*27:08* has neither 76ED nor 80TLL, but instead it has 76ES (shared with A25, A32 and Bw6-associated HLA-B antigens) and 80NLR (shared with Bw6-associated HLA-B antigens and Cw1, 3, 7, 8, 12, 14 and 16). Several non-Luminex B27 alleles (*B*27:12/18/26*, etc.) have the same combination as *B*27:08*. Other B27/B37/B47 alleles have 76ES/80NLL or 76EN/80TAL combinations. One would expect that these alleles would be acceptable mismatches for cases with 76ED/80TLL-specific antibodies.

Discussion

This report addresses the update of the ABC eplet database in the International Registry of Antibody-Defined HLA Epitopes (http://www.epregistry.com.br). Eplets are considered essential components of HLA epitopes recognized by antibodies. Their structures are based on amino acid configurations within 3 Å of polymorphic residues. The design of the new eplet repertoire consisted of three steps (1): identify all combinations of polymorphic residues within a 3-Å radius with Cn3D modeling of HLA molecular structures, (2) determine polymorphic residue compositions of 3 Å patches from amino acid sequences of HLA alleles in Luminex panels and (3) annotate eplets from one or more patches present on one allele or shared by the same group of alleles. The new HLA-ABC repertoire has 270 eplets, of which 219 are in antibody-accessible positions on the molecular surface and 51 are defined solely by residue polymorphisms located below the molecular surface, but it remains to be determined how many of the latter can elicit specific antibodies. The new repertoire is more comprehensive than the previous database in the Registry which had 233 eplets (7) and will be incorporated in the Excel programs on www.HLAMatchmaker.net and software programs such as EPHLA (9, 10) and Transplant MATCH IT™ (Immucor, LifeCodes, Stamford, CT).

This analysis often yielded multiple patches mostly in overlapping sequence positions on a given allele or shared by the same group of alleles. In such cases, one eplet was selected to annotate a group of patches. Antibodies that react only with eplet-carrying alleles in Luminex panels are considered eplet specific, but which patch they actually recognize is unknown. From specific antibody reactivity patterns, one can readily determine mismatch acceptability for Luminex alleles. As illustrated by the four examples shown above, such assessments might be more challenging for non-Luminex alleles. For each eplet, the Registry has a list of Luminex and non-Luminex alleles for which mismatch acceptability can be readily determined. However, for some non-Luminex alleles, it will be impossible to ascertain their eplet-based mismatch acceptability unless informative alleles have been added to the Luminex kits for antibody testing.

The HLA-ABC eplets recorded in the Registry are limited to those on Luminex alleles. Non-Luminex alleles may have additional eplets, but they are not included because they would be undetectable with current Luminex kits for antibody testing. It has not been determined which of these eplets might be relevant in the clinical setting; this depends on the frequencies of eplet-carrying alleles in the donor population at a transplant center.

The HLA-ABC eplet database should be considered as a resource of structurally defined epitopes recognized by HLA alloantibodies. An important question is which of these epitopes can be verified experimentally with specific antibodies. This issue of *Tissue Antigens* has the first report of antibody-defined HLA-ABC epitopes verified so far (11).

Conflict of interests

The author has declared no conflicting interests.

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