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First report on the antibody verification of HLA-ABC epitopes recorded in the website-based HLA Epitope Registry

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Abstract

The International Registry of Antibody-Defined HLA Epitopes (http://www.epregistry.com.br) has been recently established as a tool to understand humoral responses to human leukocyte antigen (HLA) mismatches. These epitopes are defined structurally by three-dimensional molecular modeling and amino acid sequence differences between HLA antigens. So-called eplets represent essential components of HLA epitopes and they are defined by polymorphic residues. A major goal is to identify HLA epitopes that have been verified experimentally with informative antibodies. Our analysis has also included data in many publications. As of 1 November 2013, 95 HLA-ABC antibody-verified epitopes have been recorded, 62 correspond to eplets and 33 are defined by eplets paired with other residue configurations. The Registry is still a work-in-progress and will become a useful resource for HLA professionals interested in histocompatibility testing at the epitope level and investigating antibody responses to HLA mismatches in transplant patients.

Introduction

The identification of human leukocyte antigen (HLA) epitopes recognized by antibodies is important for determining HLA mismatch acceptability for sensitized transplant patients and for a better understanding of humoral sensitization to HLA. The elucidation of three-dimensional molecular structures and amino acid sequence differences between HLA antigens has made it possible to define the structural basis of HLA epitopes. Under auspices of the 16th International Histocompatibility and Immunogenetics Workshop, we have developed a website (http://www.epregistry.com.br) for the International Registry of Antibody-Defined HLA Epitopes (1).

The goal of the Registry is to develop a repertoire of HLA epitopes that have been verified by specific antibodies. During the past 30 years, a considerable literature has emerged about antibody-defined HLA epitopes and many different methods have been used to define them including Luminex-based assays, flow cytometry testing, enzyme-linked immunosorbent assay (ELISA) procedures and complement-dependent lymphocytotoxicity assays. A significant limitation is that certain antibody reactivity patterns are technique-dependent and this might lead to different conclusions about the specific recognition of epitopes. We would prefer more sensitive methods, and the HLA panels used for antibody testing must be informative enough for epitope specificity analysis. Epitope-specific antibodies should be monospecific. Human monoclonal antibodies (mAbs) are ideal especially if HLA information is available for antibody producer and immunizer. Sera from sensitized persons may have informative epitope-specific antibodies, but absorption–elution studies with selected single alleles might be necessary to ascertain monospecificity. Mouse monoclonal antibodies might be useful, but we must consider the fact that they recognize xenoepitopes that might be different from alloepitopes.

The HLA Epitope Registry consists of five epitope databases: HLA-ABC, HLA-DRB, HLA-DQ, HLA-DP and MICA (1). Each database has a list of potential epitopes defined by small configurations of polymorphic amino acid residues called eplets that have been sorted according to their sequence locations on the molecular surface. This is the first report on antibody-verified HLA-ABC epitopes recorded in the Registry. Epitope-specific antibody reactivity data have been summarized from many publications during the past 25 years as well as Luminex reactivity patterns with single

alleles observed in our research laboratories. Other papers address antibody-verified HLA class II and MICA epitopes in the Registry (2, 3).

Materials and methods

Databases in the HLA Epitope Registry

Each of the five epitope databases in the Registry has the following design: (1) a list of single structural clusters of amino acid residues (eplets) determined by molecular modeling as potential epitopes recognized by alloantibodies. Eplet names have distinct sequence position numbers and residue descriptions with standard single letter amino acid codes. Antibody-verified variants such as eplet pairs or other molecular configurations are listed in separate rows under corresponding epitopes. (2) Each epitope has a description of polymorphic residues and their sequence positions. (3) Epitope frequencies calculated from HLA allele frequencies in representative population groups. (4) Antibody-verified epitopes can be classified as 'confirmed' or 'provisional' depending on the amount of information available in terms of how often specific antibodies have been identified and the completeness of reactivity patterns with informative panels. Special consideration has been given to allosensitization-induced human mAbs and the reactivity of eluates of selectively absorbed allosera as well as the antibody reactivity with mutated alleles. Future studies may permit upgrades from 'provisional' to 'confirmed' status if additional experimental support becomes available. Conversely, there could be downgrades to 'questionable' status if new data contradict previous interpretations. (5) Polymorphic residue information about eplet-related 'structural' epitopes on eplet-carrying alleles. Certain residue configurations are important binding sites with antibody, and they can be identified because they are shared between antibody-reactive alleles. (6) Epitope-carrying alleles in Luminex panels. This includes all potential and antibody-verified epitopes. (7) Listing of all alleles for each antibody-verified epitope. This includes all four-digit alleles with expressed proteins as recorded in recent HLA Nomenclature reports.

Each epitope database has a webpage with search options to identify selected repertoires of antibody-verified or potential epitopes: (1) the epitope repertoire of a selected allele and (2) all mismatched epitopes for a given HLA phenotype. These features can also be combined to identify epitopes on a donor allele that are mismatched for a recipient's HLA type.

Verification of antibody-defined HLA epitopes

This report presents a list of antibody-verified epitopes in the recently updated HLA-ABC database as described in another report in *Tissue Antigens* (4). The http://www.epregistry.com.br website describes the reactivity patterns of epitope-specific antibodies tested by the authors of this report with Luminex assays using single alleles. Our analysis included also a large collection of papers published during the past 30 years. Especially, El-Awar and Terasaki have made major contributions in this area (5–8). Epitope reactivity has been determined with informative antibodies tested with HLA panels in antibody-binding assays such as Luminex and flow cytometry and by complement-dependent lymphocytotoxicity. Most antibody reactivity patterns described in early publications were determined with smaller HLA panels, and the methodologies were less sensitive than Luminex assays with single alleles now widely available. Fortunately, few studies included gene manipulation approaches such as site mutagenesis resulting in alleles with informative amino acid residue substitutions that have provided convincing evidence about the structural basis of certain antibody-defined HLA epitopes. All antibody-verified epitopes reported here are related to allosensitization events. We have excluded the so-called natural epitopes detected by antibodies in sera from non-transfused normal males and uniquely expressed by certain class I alleles (9).

Results

As of 1 November 2013, a total of 95 antibody-verified HLA-ABC epitopes has been recorded in the Registry. In this report, listings of epitope-carrying alleles are restricted to those generally used in Luminex antibody testing kits produced by commercial vendors. Table 1 shows how HLA antigens are related to these Luminex alleles. Many antigens such as A1 and A3 correspond to one allele, whereas other antigens such as A2 and A11 include two or more alleles.

Most antibody-verified epitopes are equivalent to eplets, whereas others correspond to variants defined by eplets paired with amino acid configurations in different molecular locations. Tables 2 and 3 have five columns showing (1) epitope annotations and status (confirmed or provisional as indicated by an asterisk), (2) polymorphic residue descriptions, (3) sources of specific antibodies (H, human monoclonal antibody; M, mouse monoclonal antibody; A, alloserum; E, eluate of absorbed alloserum), (4) descriptions of epitope-carrying antigens; alleles are displayed if others in the antigen group lack the epitope and (5) lists of cited publications. The http://www.epregistry.com.br website provides more detailed documentation about antibody-verified epitopes and their expression on Luminex and non-Luminex alleles.

Antibody-verified HLA-ABC epitopes that correspond to eplets

Table 2 is a listing of 62 antibody-verified HLA-ABC epitopes that are equivalent to eplets. These eplets reside in sequence positions 14–267. As described in the accompanying paper (4), some eplets like $44KM_3$ and $62GK_2$ have a subscripted number; this indicates two or more distinct

Table 1 HLA-ABC antigens and their corresponding alleles used in Luminex kits manufactured by commercial vendors

Ag	Alleles	Ag	Alleles	Ag	Alleles	Ag	Alleles
A1	*A*01:01*	B7	*B*07:02/03*	B53	*B*53:01*	B78	*B*78:01*
A2	*A*02:01/02/03/05/06*	B8	*B*08:01*	B54	*B*54:01*	B81	*B*81:01*
A3	*A*03:01*	B13	*B*13:01/02*	B55	*B*55:01*	B82	*B*82:01/02*
A11	*A*11:01/02*	B14	*B*14:01/02/05/06*	B56	*B*56:01*	Cw1	*C*01:02*
A23	*A*23:01/02*	B18	*B*18:01*	B57	*B*57:01/03*	Cw2	*C*02:02/10*
A24	*A*24:02/03*	B27	*B*27:03/05/08*	B58	*B*58:01*	Cw3	*C*03:02/03/04*
A25	*A*25:01*	B35	*B*35:01/08*	B59	*B*59:01*	Cw4	*C*04:01/03*
A26	*A*26:01*	B37	*B*37:01*	B60	*B*40:01*	Cw5	*C*05:01*
A29	*A*29:01/02*	B38	*B*38:01*	B61	*B*40:02/06*	Cw6	*C*06:02*
A30	*A*30:01/02*	B39	*B*39:01/05*	B62	*B*15:01*	Cw7	*C*07:01/02/04*
A31	*A*31:01*	B41	*B*41:01*	B63	*B*15:16*	Cw8	*C*08:01/02*
A32	*A*32:01*	B42	*B*42:01*	B64	*B*14:01*	Cw12	*C*12:02/03*
A33	*A*33:01/03*	B44	*B*44:02/03*	B65	*B*14:02*	Cw14	*C*14:02*
A34	*A*34:01/02*	B45	*B*45:01*	B67	*B*67:01*	Cw15	*C*15:02*
A36	*A*36:01*	B46	*B*46:01*	B71	*B*15:10/18*	Cw16	*C*16:01*
A43	*A*43:01*	B47	*B*47:01*	B72	*B*15:03*	Cw17	*C*17:01*
A66	*A*66:01/02*	B48	*B*48:01*	B73	*B*73:01*	Cw18	*C*18:01/02*
A68	*A*68:01/02*	B49	*B*49:01*	B75	*B*15:02/11*		
A69	*A*69:01*	B50	*B*50:01*	B76	*B*15:12*		
A74	*A*74:01*	B51	*B*51:01/02*	B77	*B*15:13*		
A80	*A*80:01*	B52	*B*52:01*				

residue configurations on the same allele(s). Several of them correspond to well-defined serologic HLA specificities identified by lymphocytotoxicity-based typing such as 163RG (A1), $62GK_2$ (A2), 161D (A3) and 151AHA (A11). Others are shared between two antigens; examples are $44KM_3$ (A1 and A36), 56R (A30 and A31), 62GRN (B57 and B58), 62LQ (A29 and A43), 65GK (A23 and A24), 76ESI (A25 and A32) and 107W (A2 and A69). Specific antibodies against such eplets and others like 144TKH, 149TAH and 158T have often been used as serological typing reagents.

The majority of antibody-verified eplets are shared by multiple antigens, which have been traditionally classified as the so-called cross-reacting groups (CREGs). Each CREG has several epitopes in distinct molecular locations (10, 11). For instance, the A2-CREG that consists of A2, A23, A24, A68 and A69 shares a collection of antibody-verified eplets that include $62GK_2$, 65GK, 107W, 127K, 144TKH and 145KHA. It should be noted that the 127K eplet fully reflects the A2-CREG. On the other hand, A2 can also share eplets with other groups of antigens, e.g. 144K (on A1, A2, A3, A11, A24, A36, A68, A69, A80), 150AAH (on A2 except *A*02:03*, A3, A11, A24, A68 and A69) and 253Q (on A2, A25, A26, A29, A31, A32, A33, A34, A43, A66, A68, A69, A74, B73, Cw7, Cw17).

The serological Bw4/Bw6 system was originally identified by van Rood as 4a/4b (12). These so-called public antigenic determinants are shared by non-overlapping HLA-B antigen groups, and they are structurally defined by amino acid differences in sequence positions 79–83. Most antibody-verified epitopes correspond to eplets but in many cases their structural description is more complex. All Bw4-carrying HLA-B antigens as well as A23, A24, A25 and A32 share the 82LR eplet defined by 79R82L83R. Site mutagenesis studies by Lutz et al. (13) have demonstrated the importance of specific residues in the 79–83 sequence, but mutations in these positions had different effects on the reactivity of Bw4-specific antibodies, thereby suggesting heterogeneity within Bw4 epitopes. Bw4 has three variations in the 79–83 sequence RIALR (identical to 80I on A23, A24, A25, A32, B5, B38, B49, B53, B57, B58, B59, B63 and B77), RTALR (identical to 80TAL on B13 and B44) and RTTLR (identical to 76ED on *B*27:03*, *B*27:05*, B37 and B47). Table 2 lists 80I as antibody-verified, but insufficient data are available for 76ED and 80TA.

Two Bw6-associated eplets 76ESN and 80N have also been antibody verified. The 80N eplet is defined by the 79R80N82R83G sequence and is present on all Bw6-carrying HLA-B alleles, but it should be noted that this antibody-verified eplet is also present on a group of HLA-C antigens.

HLA-C encodes several antibody-verified eplets; some are present in one or two antigens, i.e. 173K (on Cw3), $193PL_3$ (on Cw7) and 138K (on Cw5 and Cw8). Others like 21H, 80K, 177KT and 219W are shared by larger groups of HLA-C antigens. It should be noted that several antibody-verified eplets are shared between groups of antigens encoded by two or three loci, e.g. 62GE, 62RR, 76VRN, 90D, 163EW, 166DG and 193PV.

Antibody-verified HLA-ABC epitopes defined by eplets paired with other residue configurations

Many antibodies recognize epitopes that correspond to specific eplets, but certain eplet-carrying antigens are non-reactive.

Table 2 Antibody-verified HLA-ABC epitopes which correspond to eplets

HLA-A,B,C epitope (*, provisional)	Polymorphic residue description	Epitope-carrying antigens and/or alleles in Luminex kits	Antibody source	References
17RS*	14R17S	A30	A	(7)
21H	21H	Cw2,Cw3,*C*04:03*,Cw15	A, E	(7, 29, 30)
41T	41T	B13,B40,B41,B44,B45,B47,B49,B50	H, E	(5, 31, 32)
$44KM_3$	44K45M (149A150V151H152A) (158V)	A1,A36	M	(5, 31, 33)
44RMA	44R45M46A	B13,B46,B57,B62,B63,B75,B76,B77	M	(8)
44RT*	44R45T46E	B18,B35,B37,B51,B52,B53,B58,B78	M	(7)
56R	56R	A30,A31	A, M	(7, 8, 33–35)
62EE*	62E63E	A23,A24,A80	H, M	(7, 31)
62GE	62G63E65R	A2,B57,B58	H, E, M	(5, 33, 36–38)
$62GK_2$	62G63E65R66K(73T74H76V77D)	A2	M	(5, 31, 33)
62GRN	62G63E65R66N	B57,B58	H, M	(7, 33, 39)
62LQ	62L63Q65R66N	A29,A43	H, M	(5, 33, 40)
62RNR*	62R63N65R	A25,A26,A33,A34,A66,A68,A69	E	(5)
62RR*	62R65R	A25,A26,A33,A34,A66,A68,A69,B63	M	(7, 41)
65GK	62E63E65G66K69A	A23,A24	H, M	(5, 34, 35)
65QIA	65Q66I69A	*B*07:02*,B27,B42,B54,B55,B56,B67, B73,B81,B82	H, E, M	(5, 33, 42)
69AA	69A71	*B*07:02*,B27,B42,B54,B55,B56,B57,B58, B63,B67,B73,B81,B82	E	(5, 32)
70IAQ*	66I69A70Q71A	*B*07:02*,B42,B54,B55,B56,B67,B81,B82	E	(5, 41)
69TNT*	69T70N71T	*B*07:03*,B8,B13,B14,B62,B75,B76, B77,B70,B18,B35,B38,B39, B41,B44,B45,B47,B48,B49,B50,B51, B52,B53,B59,B60,B61,B64,B65,B78	E	(5, 41)
71SA	66N69A70S71A73T	B63,B57,B58	M	(5, 41)
73TVS*	69R73T76V77S	B46, Cw1,Cw3,Cw8,Cw14,Cw16	E	(7)
71TTS*	71T73T77S	*B*07:03*,B8,B14,B18,B35,B39,B41,B45, B48,B50,B60,B61,B62,B64,B65,B70,B75,B76,B78	H	(43)
76ANT	76A77N79G80T	A1,A26,A29,A36,A43,A80	E	(5)
76ESI	76E77S79R80I	A25,A32	M	(31, 34, 35, 42, 44)
76ESN	76E77S79R80N	B7,B8,B14,B18,*B*27:08*,B35,B39, B41,B42,B45,B48,B50,B54,B55,B56,B60,B61, B62,B64,B65,B67,B70,B75,B76,B78,B81,B82	M, E	(5, 13)
76VRN*	76V79R80N	B46,B73, Cw1,Cw3,Cw7,Cw8, Cw12,Cw14,Cw16	A	(7, 30)
80TLR*	76E79R80T82L83R	B13,*B*27:03/05*,B37,B44,B47	E	(5)
80I	79R80I81A82L83R	A23,A24,A25,A32, B38,B49,B51,B52, B53,B57,B58,B59,B63,B77	H, E	(5, 32)
80K	76V77N79R80K81L82R83G	Cw2,Cw4,Cw5,Cw6,Cw15,Cw17, Cw18	A, E	(7, 29, 30, 45)
80N	79R80N82R83G	B7,B8,B14,B18,*B*27:08*,B35,B39, B41,B42,B45,B46,B48,B50,B54,B55, B56,B60,B61,B62,B67,B70,B73,B75,B76,B78, B81,B82,Cw1,Cw3,Cw7,Cw8, Cw12,Cw14,Cw16	H, E	(5, 32, 42)
82LR	79R82L83R	A23,A24,A25,A32,B13,*B*27:03/05*,B37,B38, B44,B47,B49,B51,B52,B53,B57, B58,B59,B63,B77	H, E, M	(5, 13, 32, 33, 35, 46)
90D*	90D91G	A1,A11,A25,A26,A34,A36,A43,*A*66:01*,A80, B73, Cw4,Cw6,Cw7,Cw18	E	(5, 41)
107W	107W	A2,A69	M	(33–35, 37, 38, 47, 48)
127K	127K	A2,A23,A24,A68,A69	A, M, E	(5, 8, 38, 49)
138K*	138K	Cw5,Cw8	A	(30)
144K*	144K	A1,A2,A3,A11,A24,A36,A68,A69,A80	E	(5, 41)
144TKH	142T143T144K145H	A2,A68,A69	A, M, H, E	(5, 33, 42)
144KR	142I143T144K145R	A1,A3,A11,A24,A36,A80	H, E	(5, 31)
145KHA	144K145H149A	A2 (not *A*02:03*),A68,A69	M, H	(31, 33, 50, 51)
144QL	142I143T144Q145L	B13	A, H, M	(31, 33–35, 39, 47)
145RT*	145R149T	A25,A26,A34,A43,A66	M	(5, 52)
150AAH	149A150A151H	A2(not *A*02:03*),A3,A11,A24,A68,A69	M	(8)
149TAH	149T150A151H	*A*02:03*,A25,A26,A34,A43,A66	A	(7)
151AHA	150A151H152A	A11	H, A	(7)
152RW*	151R152W	*A*30:01*	H	(53)

Table 2 Continued

HLA-A,B,C epitope (*, provisional)	Polymorphic residue description	Epitope-carrying antigens and/or alleles in Luminex kits	Antibody source	References
156DA*	156D158A	B8,B37,B41,B42,*B*44:02*,B45,B82, *C*07:04*	A	(54)
158T	158T	B38,B39,B67	H, M	(5, 55)
161D	161D	A3	A, H, M	(5, 21, 33–35, 52)
163EW	162G163E166E167W	*A*66:02*, B7,B13,B27,B47,B48,B60, B61,B73,B81, Cw2,Cw17	E	(5)
163LW	162G163L166E167W	B35,B46,B49,B50,B51,B52,B53,B56,B57,B58, B62,B63,B70, B75,B77,B78, Cw3	H, E	(7, 29, 31, 32, 39)
163R*	163R	A1,A11,A25,A26,A43,*A*66:01*	E	(5, 41, 49)
163RG	162G163R166D167G	A1	H, M	(31)
163RW	162G163R166E167W	A11,A25,A26,A43,*A*66:01*	H, E	(5, 31, 56)
166DG	166D167G	A1,A23,*A*24:02*,A80, B76	E, M	(5, 31, 33, 41)
173K	173K	Cw3	A	(29, 30)
177KT*	177K178T	Cw5,*C*07:04*,Cw8	A	(7, 30)
180E	180E	B7,B8,B41,B42,B48,B60,B81	M, E	(5, 57–59)
$193PL_3$	193P194L(182A183E184P)(70S)	Cw7	E	(7, 29, 30)
193PV*	193P194V	B35,B51,B52,B53,B58,B78,Cw1, Cw2,Cw3,Cw4,Cw5,Cw6,Cw8,Cw12,Cw14,Cw15, Cw17,Cw18	A	(30, 52)
219W	219W	Cw1,Cw3,Cw4,Cw14,Cw18	H	(25, 30, 31)
253Q*	253Q	A2,A25,A26,A29,A31,A32,A33,A34,A43,A66,A68, A69,A74, B73, Cw7,Cw17	E	(7, 29, 41)
267QE*	267Q268E	B73,Cw7,Cw17	M	(29, 41)

A, alloserum; E, eluate of absorbed alloserum; H, human monoclonal antibody; M, mouse monoclonal antibody.

These findings suggest that reactive eplet-carrying alleles must have additional configurations necessary for binding with antibody. Such configurations might be identified by applying the concept that an eplet is part of a structural HLA epitope that makes contact with the six complementarity determining regions (CDRs) of antibody (14, 15). Molecular modeling of protein antigen–antibody complexes has demonstrated that the so-called structural epitopes have 15–25 antibody-contacting residues distributed over a molecular surface area of 700–900 $Å^2$ (16, 17). Besides the centrally located so-called functional epitope that dominates specific antibody recognition, other antibody-contacting residues in the corresponding structural epitope have been shown to play a critical role in antibody binding (18, 19). These findings may also apply to HLA epitopes whereby eplets are considered equivalent to functional epitopes, and certain amino acid configurations within corresponding structural epitopes, i.e. within a 15 Å radius of an eplet, would serve as critical contact sites for antibody (20). Indeed, human monoclonal antibodies have demonstrated HLA-ABC epitopes defined by eplets paired with other nearby amino acid configurations (21, 22). In each case, the eplet was non-self on the immunizing allele and the paired configuration consisted of self-residues present in the antibody producer and also the immunizer.

Table 3 lists 33 epitopes defined by pairs which have been verified with human monoclonals and other informative antibodies; all of them have a provisional status because more definitive studies need to be done. These pairs always involve amino acid configurations located on the molecular surface between 5 and 15 Å from each other, thereby suggesting the binding with at least two separate CDRs of antibody. These pairs often comprise two eplets listed in the Registry. For instance, Table 2 has the antibody-verified epitope 44RT shared by B18, B35, B37, B51, B52, B53, B58 and B78. Table 3 has 44RT + 69TNT expressed by all these antigens except B58 that has 44RT + 69ASA. At this time, there is no antibody verification of 62QE that is shared between A1, A3, A11, A30, A31, A32, A36 and A74, but there are two antibody-verified 62QE-related epitopes defined by pairs: 62QE + 56G is on all 62QE-expressing alleles except A30 and A31 that have 62QE + 56R, whereas 62QE + 151H is on A1, A3, A11 and A36 but not on A30, A30, A32 and A74 that have 62QE + 151R.

Table 3 has also antibody-verified epitopes defined by eplets paired with locus-specific monomorphic residues. These eplets are shared between antigens encoded by two or more loci. For instance, 90D is shared between A1, A11, A25, A26, A34, A36, A43, *A*66:01* (not *A*66:02*), A80, B73, Cw4, Cw6, Cw7 and Cw18. The antibody-verified 90D + 138M epitope has the monomorphic methionine residue that is restricted to HLA-A, whereas B73 and 90D-carrying HLA-C antigens have 90D + 138T.

The verification of pair-specific epitopes provides further documentation of the complexity of the antibody response to a mismatched eplet. The Bw4-associated and antibody-verified epitopes 80I and 82LR offer informative illustrations. The 82LR eplet is shared between A23, A24, A25, A32, B13, *B*27:03/05*, B37, B38, B44, B47, B49, B51, B52, B53, B57,

Table 3 Antibody-verified HLA-ABC epitopes defined by eplets paired with other residue configurations

HLA-A,B,C epitope (*, provisional)	Polymorphic residue description	Epitope-carrying antigens and alleles in Luminex kits	Antibody source	References
44RT + 69TNT*	44R45T46E paired with 69T70N71T	B18,B35,B37,B51,B52,B53,B78	E	(41, 60)
62QE + 56G*	62Q63E65R66N paired with self 56G	A1,A3,A11,A32,A36,A74	H	(21, 31, 39)
62QE + 151H*	62Q63E65R66N paired with self 151H	A1,A3,A11,A36	M, H	(8, 22)
62RN + 163TW*	62R63N paired with 163T166W	A33,A34,A68,A69;B8,B14,B18,B38, B39,B42,B54,B55,B59,B64,B65,B67	A	(7, 41)
65QIA + 76ESN*	65Q66I69A paired with self 76E77S80N	*B*07:02*, **27:08*,B42,B54,B55,B56,B67,B81,B82	H	(22)
65QK + 76VS*	62R65Q66K67Y69R paired with self 76VS	B46, Cw1,Cw3,*C*07:02*, **07:04*,Cw8, Cw12,Cw14,Cw16	H	(43)
65RNA + 80I*	65R66N69A paired with self 80I82L83R	A25,A32,B57,B58,B63	H	(22, 31)
66IF + 163TW*	66I67F69T paired with 163T166W	B8,B59	M, E	(5, 8, 41)
69AA + 80N*	69A71A paired with self 80N	*B*07:02*,B27:08,B42,B54,B55,B56,B67,B73,B81,B82	H	(32)
69TNT + 80N*	69T70N71T paired with 80N	*B*07:03*,B8,B14,B18,B35,B39,B41,B45,B48, B50,B60,B61,B62,B64, B65,B70,B75,B76,B78	E	(5, 41)
76ENI + 62RE*	76E77N79R80I paired with 62R63E	B49,B52,B63	A	(7, 41)
76VS + 152RE*	76V77S80N paired with 151R152E	B46,Cw1,Cw3,*C*08:02*,Cw12,Cw14	A	(30)
79GT + 19E*	79G80T paired with 19E	A1,A2,A3,*A*11:01*,A26,A29,A30,A31, A33,A34.A36,A43,A66,A68,A69,A74,A80	M	(7, 41)
80I + 65QI*	79R80I81A82L83R paired with 65Q66I	B38,B49,B51,B52,B53,B59,B77	A	(5, 41)
80I + 90A*	79R80I81A82L83R paired with 90A	A23,A24,A32,B38,B49,B51,B52,B53, B57,B58,B59,B63,B77	E	(5, 41)
80I + 152RE*	79R80I81A82L83R paired with 151R152E	B49,B51,B52,B77	M	(5, 41)
82LR + 90A*	79R82L83R paired with 90A	A23,A24,A32,B13,*B*27:03/05*,B37, B38,B44,B47,B49,B51,B52,B53,B57, B58,B59,B63,B77 (not A25)	M	(8, 33–35, 61)
82LR + 138M*	79R82L83R paired with 138M	A23,A24,A25,A32	M	(33)
82LR + 138T*	79R82L83R paired with 138T	B13,*B*27:03/05*,B37,B38,B44,B47,B49,B51,B52,B53, B57,B58,B59,B63,B77	M	(13, 33)
82LR + 144QR*	79R82L83R paired with 144Q145R	A23,A25,A32,*B*27:03*, **27:05*,B37, B38,B44,B47,B49,B51,B52,B53,B57, B58,B59,B63,B77 (not A24 and B13)	A, M	(8, 13)
82LR + 145R*	79R82L83Rpaired with 145R	A23,A24,A25,A32,*B*27:03*, **27:05*,B37,B38,B44,B47,B49, B51,B52,B53,B57,B58,B59,B63,B77 (not B13)	M	(8)
82LR + 145RA*	79R82L83R paired with self 145R149A	A23,A24,A32,*B*27:03*, **27:05*,B37,B38, B44,B47,B49,B51,B52,B53,B57, B58,B59,B63,B77 (not A25 and B13)	H	(22, 62)
90D + 138M*	90D91G paired with 138M	A1,A11,A25,A26,A34,A36,A43, *A*66:01*, A80	E	(5, 41, 49)
131S + 163LW*	131S paired with 163L166W	B35,B46,B49,B50,B51,B52, B53,B56,B57,B58,B62,B63,B70,B75, B77,B78	E	(5, 41)
131S + 163T*	131S paired with 163T166W	B14,B18,B37,B38,B39,B54,B55,B59, B64,B65,B67	E	(5, 41)
138MI + 79GT*	138M142I143T145R paired with self 79G80T	A1,A3,A11,A26,A29,A30,A31,A33, A34,A36,A43,A66,A74,A80	H	(21, 22, 31)
143S + *76*ESN*	143S paired with self 76ESN	B48,B60,B81	H	(39)
144K + 76VDT*	144K paired with 76V77D79G80T	A2,A3,A11,A68,A69	E	(5, 41)
144KR + 151H*	142I143T144K145R paired with self 151H	A1,A3,A11,A24,A36	H	(21, 22)
163EW + 73TE*	162G163E166E167W paired with self 73T76E	B7,B13,B27,B47,B48,B60,B61,B81	A, H	(22) (5)
163LW + 65QI*	162G163L166E167Wpaired with self 65Q66I	B35,B49,B50,B51,B52,B53,B56, B62,B63,B70,B75,B77,B78	H	(22, 32)
163TEW + 65QI*	163T166E167W paired with 65Q66I	B8,B14,B18,B37,B38,B39,B41,B42, B54,B55,B59,B64,B65,B67	A	(7, 30)
163TEW + 103L*	163T166E167W paired with 103L	B54,55,59; Cw1,Cw4,Cw5,Cw6,Cw7,Cw8,Cw12,Cw14, Cw15,Cw16,Cw18	M	(7, 30)

[a]A, alloserum; E, eluate of absorbed alloserum; H, human monoclonal antibody; M, mouse monoclonal antibody.

B58, B59, B63 and B77. Six related but distinct epitopes correspond to 82LR paired with other residues outside the 79–83 sequence (Table 3). Each one is on 82LR-carrying antigens but not all of them: 82LR + 90A (not on A25 that has 90D), 82LR + 138M (only on 82LR-carrying HLA-A antigens), 82LR + 138T (only on 82LR-carrying HLA-B antigens), 82LR + 144QR (not on A24 that has 144KR and on B13 that has 144QL), 82LR + 145R (not on B13 that has 145L) and 82LR + 145RA (not on A25 that has 145RT and on B13 that has 145LA).

The antibody-verified 80I eplet is on a different group of Bw4-associated antigens, namely A23, A24, A25, A32,

B38, B49, B51, B52, B53, B57, B58, B59, B63 and B77. Three 80I-related epitopes have been verified (Table 3). The 80I + 65QI epitope is expressed by all 80I-carrying antigens except A23, A24, A25, A32, B57, B58 and B63 that have 65GK or 65RNA. The 80I + 90A pair is absent on A25 that has 80I + 90D, whereas 80I + 152RE is only expressed on B49, B51, B52, B63 and B77.

It should be noted that several pairs were identified with human monoclonal antibodies for which HLA typing information of the immunizer and antibody producer was available. In each case, the non-self eplet is paired with a self-configuration shared between the antibody producer and immunizer (see Table 3, second column). Examples are 62QE + (self)56G and the Bw4-associated 82LR + (self)145RA. Bw4-associated sequences may also operate as self-configurations in eplet pairs, e.g. 65RN + (self)80I and 143S + (self)76ESN.

Discussion

This report describes 95 antibody-verified HLA-ABC epitopes including 33 defined by combinations of amino acid configurations. Such epitopes are expressed by either one antigen or small groups or large groups of antigens encoded by one or more loci. The verification of an antibody-defined epitope depends on the antibody reactivity pattern with an informative HLA panel, but this is not always a simple matter. In contrast to the nomenclature of HLA alleles, which is solely based on nucleotide or amino acid differences in sequence positions, the criteria for HLA epitopes are different because they are based on physiochemical interactions between antibodies and HLA antigens. We assume that HLA antibody-specific epitopes can be defined by distinct amino acid configurations involving non-self polymorphic residues on the molecular surface. Small configurations are called eplets and indeed, there are many antibody-verified epitopes that correspond to eplets. Many such epitopes have been identified in Luminex assays with single alleles, and we have also included data using different antibody reactivity assays published by many investigators as well as progress reports of international HLA workshops.

HLA polymorphisms also involve residues in positions below the molecular surface and which cannot make direct contact with antibody. Hidden residues can influence the conformation of nearby polymorphic residues on the molecular surface, thereby creating distinct epitopes. For instance, 62RR (62R65R) and 62RNR (62R63N65R) are two antibody-verified epitopes (Table 2). They reflect structurally similar eplets except that one requires the participation of the hidden 63N. One might raise the question whether hidden residues influence the conformation of monomorphic residue configurations on the molecular surface, thereby giving rise to epitopes that could be recognized by antibodies. At present, we have not seen experimental verification of such epitopes.

Many antibody-verified epitopes correspond to eplets paired with other distinct configurations located close enough on the molecular surface to be contacted by different CDRs of antibody. These findings demonstrate that HLA epitopes are structurally complex. Molecular modeling of protein antigen–antibody complexes has shown that each heavy and light chain of antibody has three CDRs that bind up to 15–25 residues distributed over a 700–900 $Å^2$ surface of a so-called structural epitope (16, 17). One heavy chain CDR (mostly CDR-H3) interacts with a so-called functional epitope and dominates the determination of antibody specificity, whereas one or two other CDRs make critical contributions to the strength of antibody binding to epitopes.

These concepts must also apply to HLA antibodies. Eplets correspond to functional epitopes and they may pair with other configurations to generate distinct epitopes. From the HLA typing information of the immunizing event, it appears that these configurations are always shared between the antibody producer and the immunizing allele (21, 22). In other words, many HLA epitopes are defined by non-self eplets paired with self-HLA components of antibody producers. Subsequent studies have shown that within the context of structural epitopes, virtually every surface residue within 15 Å of a non-self eplet on the immunizing antigen is self for the antibody producer (23). These findings have led to the non-self–self paradigm of HLA epitope immunogenicity whereby antibodies are believed to have originated from B-cells with receptors for self-HLA epitopes. Such B-cells cannot respond to self-eplets. On the other hand, non-self eplets surrounded by self-residues could activate B-cells and trigger antibody responses.

These concepts may offer some understanding on why so many epitopes are structurally related. The Bw4-associated, antibody-verified 80I and 82LR eplets are typical examples. Antibodies can also be specific for 80I or 82LR eplets paired with certain polymorphic or monomorphic residue configurations (Table 3). Why would such configurations be so important especially if they are self for the antibody producer? The answer to this question must be related to the process of affinity maturation that affects every B-lymphocyte transformed into an antibody-producing cell. Affinity maturation is the result of somatic mutations of immunoglobulin genes of dividing B-cells; this alters the residue compositions of CDRs to increase the binding strength of antibodies (24). These residue substitutions may enhance the specificity-determining CDRs to functional epitopes and especially they will increase the affinity of the remaining CDRs to other parts of structural epitopes. As structural HLA epitopes consist of non-self eplets surrounded largely by self-residues, it seems likely that some self-configurations will have increased binding abilities with antibodies that have undergone affinity maturation. For the Bw4-associated epitopes, such configurations have been shown to locate in sequence positions 65–66, 90, 138, 144–149 and 151–152 (see Table 3). They are all within a 15-Å radius of 80I and 82LR.

In conclusion, eplet-carrying alleles in HLA panels may have corresponding structural epitope differences that

affect their reactivity with antibody. This concept may also explain differences in antibody reactivity patterns determined in immunoglobulin-binding assays and the less sensitive complement-dependent lymphocytotoxicity methods (25). They can lead to different interpretations of epitopes recognized by antibodies, and this may limit the usefulness of the less sensitive lymphocytotoxicity assays. On the other hand, one might also argue that epitopes verified with lymphocytotoxic antibodies would be clinically more relevant because complement-dependent antibodies are important in transplant rejection.

The antibody verification of HLA epitopes has another limitation in that certain class I reactive antibodies are peptide-dependent (26–28). This means that their CDRs make critical contacts with the residues of the peptides that bound to the groove. This situation would apply to antibodies recognizing eplets on the α-helical structures next to the peptides. Under natural conditions, each HLA allele binds thousands of different peptides and varying proportions would have the necessary contact residues, and this would affect the degree of binding with antibody. This concept might explain why certain epitope-carrying alleles have rather low antibody reactivity in Luminex assays, which cannot be explained by residue differences between the HLA chains themselves. It is possible that the same allele in Luminex kits prepared by the different commercial vendors may not have the same repertoire of bound peptides, and this could affect their reactivity with peptide-dependent HLA antibodies. At present, there are no studies that address this issue.

At present, the repertoire of antibody-verified HLA-ABC epitopes must be considered incomplete and more information is needed. HLA professionals are invited to submit data about HLA reactive antibody reactivity patterns that will improve the repertoire of antibody-verified epitopes. These data could be generated in the laboratory, but we also welcome publications on antibody-defined epitopes, which have not been cited in this report. The Registry website http://epregistry.com.br has instructions on how to submit information about antibody reactivity patterns with HLA panels and the sensitizing event including, if possible, HLA types of antibody producer and immunizer. Moreover, additional data such as absorption/elution studies with selected alleles and the use of mutated alleles with specific residue substitutions would be helpful.

While still being a work-in-progress, the HLA Epitope Registry will become a valuable resource for HLA professionals interested in histocompatibility testing at the epitope level and investigating antibody responses to HLA mismatches.

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Conflict of interests

The authors have declared no conflicting interests.

References

1. Duquesnoy RJ, Marrari M, da Mata Sousa LCD et al. 16th IHIW: a website for the antibody-defined HLA Epitope Registry. *Int J Immunogenet* 2013: **40**: 54–9.
2. Duquesnoy RJ, Marrari M, Tambur A, da Mata Sousa LCD, do Monte SJH. First report on the antibody verification of HLA-DR, HLA-DQ and HLA-DP epitopes recorded in the HLA Epitope Registry. *Hum Immunol* 2014 (in press).
3. Duquesnoy RJ, Marrari M, Mostecki J, da Mata Sousa LCD, do Monte SJH. First report on the antibody verification of MICA epitopes recorded in the HLA Epitope Registry. *Int J Immunogenet* 2014 (in press).
4. Duquesnoy RJ. Update of the HLA class I eplet database in the website based registry of antibody-defined HLA epitopes. *Tissue Antigens* 2014: **83**: 382–90.
5. 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. *Hum Immunol* 2007: **68**: 170–80.
6. El-Awar N, Terasaki M, Cai J et al. Epitopes of HLA-A, B, C, DR, DQ, DP and MICA antigens. *Clin Transpl* 2009: **2009**: 295–321.
7. El-Awar NR, Akaza T, Terasaki PI, Nguyen A. Human leukocyte antigen class I epitopes: update to 103 total epitopes, including the C locus. *Transplantation* 2007: **84**: 532–40.
8. El-Awar NR, Terasaki PI, Nguyen A, Lias M, Conger N. New HLA class I epitopes defined by murine monoclonal antibodies. *Hum Immunol* 2010: **71**: 456–61.
9. El-Awar N, Terasaki P, Nguyen A et al. Epitopes of human leukocyte antigen class I antibodies found in sera of normal healthy males and cord blood. *Hum Immunol* 2009: **70**: 844–53.
10. Fuller AA, Rodey GE, Parham P, Fuller TC. Epitope map of the HLA-B7 CREG using affinity-purified human alloantibody probes. *Hum Immunol* 1990: **28**: 306–25.
11. Fuller AA, Trevithick JE, Rodey GE, Parham P, Fuller TC. Topographic map of the HLA-A2 CREG epitopes using human alloantibody probes. *Hum Immunol* 1990: **28**: 284–305.
12. Van Rood J, Van Leeuwen A. Leukocyte grouping. A method and its application. *J Clin Invest* 1963: **42**: 1382–90.
13. Lutz CT, Smith KD, Greazel NS et al. Bw4-reactive and Bw6-reactive antibodies recognize multiple distinct HLA structures that partially overlap in the alpha-1 helix. *J Immunol* 1994: **153**: 4099–110.
14. Kabat EA. The structural basis of antibody complementarity. *Adv Protein Chem* 1978: **32**: 1–75.
15. Chothia C, Lesk A, Tramontano A et al. Conformation of immunoglobulin hypervariable regions. *Nature* 1989: **342**: 877–83.
16. Davies D, Padlan E, Sheriff S. Antibody-antigen complexes. *Annu Rev Biochem* 1990: **59**: 439–73.
17. MacCallum RM, Martin ACR, Thornton JM. Antibody-antigen interactions: contact analysis and binding site topography. *J Mol Biol* 1996: **262**: 732–45.

18. Novotny J. Protein antigenicity: a thermodynamic approach. *Mol Immunol* 1991: **28**: 201–8.
19. Cunningham B, Wells J. Comparison of a structural and a functional epitope. *J Mol Biol* 1993: **234**: 554–63.
20. Duquesnoy RJ. A structurally based approach to determine HLA compatibility at the humoral immune level. *Hum Immunol* 2006: **67**: 847–62.
21. 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.
22. Marrari M, Mostecki J, Mulder A, Balazs I, Claas F, Duquesnoy R. Human monoclonal antibody reactivity with HLA class I epitopes defined by pairs of mismatched eplets and self eplets. *Transplantation* 2010: **90**: 1468–72.
23. Duquesnoy R. The antibody response to an HLA mismatch: a model for nonself-self discrimination in relation to HLA epitope immunogenicity. *Int J Immunogenet* 2011: **39**: 1–9.
24. Berek C, Milstein C. Mutation drift and repertoire shift in the maturation of the immune response. *Immunol Rev* 1987: **96**: 23–41.
25. Duquesnoy RJ, Marrari M, Jelenik L, Zeevi A, Claas FHJ, Mulder A. Structural aspects of HLA class I epitopes reacting with human monoclonal antibodies in Ig-binding, C1q-binding and lymphocytotoxicity assays. *Hum Immunol* 2013: **I74**: 1271–9.
26. Wang J, Yu DT, Fukazawa T et al. A monoclonal antibody that recognizes HLA-B27 in the context of peptides. *J Immunol* 1994: **152**: 1197–205.
27. Mulder M, Eijsink C, Kester MGD et al. Impact of peptides on the recognition of HLA class I molecules by human HLA antibodies. *J Immunol* 2005: **175**: 5950–7.
28. Fu X-T, Karr RW. HLA-DRα chain residues located on the outer loops are involved in nonpolymorphic and polymorphic antibody-binding epitopes. *Hum Immunol* 1994: **39**: 253–60.
29. Akaza T, El-Awar N, Nguyen A et al. HLA class I epitopes: C-locus. *Clin Transpl* 2006: **95–102**.
30. Duquesnoy R, Marrari M. Detection of antibodies against HLA-C epitopes in patients with rejected kidney transplants. *Transpl Immunol* 2011: **24**: 164–71.
31. Fernandez-Vina M, Maiers M, Gutierrez M et al. 13th IHWS serology and HLA phenotypes joint report. In: Hansen JA, ed. *Immunobiology of the Human MHC. Vojens: P.J. Schmidt A/S, 2006, 890–931.*
32. Duquesnoy R, Marrari M, Mulder A, Claas F, Mostecki J, Balazs I. Structural aspects of HLA class I epitopes detected by human monoclonal antibodies. *Hum Immunol* 2012: **73**: 267–77.
33. Tongio MM, Doxiadis I, Laforet M et al. 12th international histocompatibility workshop HLA class I monoclonal antibodies study. In: Charron D, ed. *HLA: Genetic Diversity of HLA Functional and Medical Implication. Paris: EDK, 1997, 7–11.*
34. Nelson K, Bodmer J, Martin A, Navarrete C, Strong DM. Micro EIA and monoclonal antibodies. In: Dupont B, ed. *Immunobiology of HLA. New York, NY: Springer-Verlag, 1989, 292–301.*
35. Kennedy LJ, Marsh SGE, Bodmer J. Cytotoxic monoclonal antibodies. In: Dupont B, ed. *Immunobiology of HLA. New York, NY: Springer-Verlag, 1989, 301–5.*
36. Claas F, Castelli-Visser R, Schreuder I, van Rood J. Allo-antibodies to an antigenic determinant shared by HLA-A2 and B17. *Tissue Antigens* 1982: **19**: 388–91.
37. Santos-Aguado J, Barbosa JA, Biro PA, Strominger JL. Molecular characterization of serologic recognition sites in the human HLA-A2 molecule. *J Immunol* 1988: **141**: 2811–8.
38. De Vito LD, Mason BP, Jankowska-Gan E et al. Epitope fine specificity of human anti-HLA-A2 antibodies. Identification of four epitopes including a haptenlike epitope on HLA-A2 at lysine 127. *Hum Immunol* 1993: **37**: 165–77.
39. Mulder A, Kardol M, Regan J, Buelow R, Claas F. Reactivity of twenty-two cytotoxic human monoclonal HLA antibodies towards soluble HLA class I in an enzyme-linked immunosorbent assay (PRA-STAT). *Hum Immunol* 1997: **56**: 106–13.
40. Kolstad A, Hannestad K. A supertypic HLA-DP specificity defined by two human-human hybridoma antibodies (TrB50; TrE11). *Hum Immunol* 1989: **25**: 247–56.
41. Duquesnoy R, Marrari M. Correlations between Terasaki's HLA class I epitopes and HLAMatchmaker-defined eplets on HLA-A, -B and -C antigens. *Tissue Antigens* 2009: **74**: 117–33.
42. Harpprecht J, Olde K, Westphal E, Muller-Ruchholtz W. Serological mapping of HLA-epitopes with monoclonal antibodies and its interpretation by sequenced HLA-molecules. *Tissue Antigens* 1989: **34**: 170–8.
43. Marrari M, Mulder A, Duquesnoy RJ. HLA class I epitopes defined by human monoclonal antibodies tested in Luminex assays with single alleles. 2013. http://epregistry.com.br
44. Vilella R, Yague J, Vives J. Monoclonal antibody against HLA-Aw32 + A25. Is HLA-Aw32 an allele with no unique antigenic determinant? *Hum Immunol* 1983: **6**: 53–62.
45. Bacheleta T, Couzia L, Guidicelli G et al. Anti-Cw donor-specific alloantibodies can lead to positive flow cytometry crossmatch and irreversible acute antibody-mediated rejection. *Am J Transpl* 2011: **11**: 1543–4.
46. Tahara T, Yang SY, Khan R, Abish S, Hammerling GJ, Hammerling U. HLA antibody responses in HLA class I transgenic mice. *Immunogenetics* 1990: **32**: 351–60.
47. Harpprecht J, Westphal E, Muller-Ruchholtz W. Production and applications of new monoclonal antibodies against human lymphocyte antigen-A and -B antigens. *Diagn Clin Immunol* 1988: **5**: 388–92.
48. Layet C, Kahn-Perles B, Pontarotti P, Ferrier P, Sire J, Lemonnier FA. Creation of an HLA-A2/HLA-Aw69 alloantigenic determinant on an HLA-A3 molecule by site-directed mutagenesis. *J Immunol* 1987: **138**: 2197–201.
49. Laundy GJ, Bradley BA, Rees BM, Younie M, Hows JM. Incidence and specificity of HLA antibodies in multitransfused patients with acquired aplastic anemia. *Transfusion* 2004: **44**: 814–25.
50. Hogan KT, Clayberger C, Bernhard EJ et al. Identification by site-directed mutagenesis of amino acid residues contributing to serologic and CTL-defined epitope differences between HLA-A2.1 and HLA-A2.3. *J Immunol* 1988: **141**: 2519–25.
51. Mulder A, Kardol M, Blom J, Jolley W, Melief C, Bruning H. A human monoclonal antibody, produced following in vitro

immunization, recognizing an epitope shared by HLA-A2 subtypes and HLA-A28. *Tissue Antigens* 1993: **42**: 27–34.
52. 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.
53. Pistillo MP, Bini D, Pozzi S, Ferrara GB. A cytotoxic human monoclonal antibody that can discriminate HLA-A*3002 from HLA-A*3001 subtypes. *Tissue Antigens* 1995: **46**: 327–9.
54. Lomago J, Jelenik L, Zern D et al. How did a patient who types for HLA-B*4403 develop antibodies that react with HLA-B*4402? *Hum Immunol* 2010: **71**: 176–8.
55. Ge J, Hannestad K. A cytotoxic human hybridoma monoclonal antibody (TrJ5) specific for HLA-B38(16) and -B39(16). *Hum Immunol* 1993: **36**: 168–71.
56. Mulder A, Kardol MJ, Uit het Broek CM, Tanke-Visser J, Young NT, Claas FH. A human monoclonal antibody against HLA-Cw1 and a human monoclonal antibody against an HLA-A locus determinant derived from a single uniparous female. *Tissue Antigens* 1998: **52**: 393–6.
57. McCutcheon JA, Lutz CT. Mutagenesis around residue 176 on HLA-B*0702 characterizes multiple distinct epitopes for anti-HLA antibodies. *Hum Immunol* 1992: **35**: 125–31.
58. 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.
59. Parham P. Changes in conformation with loss of alloantigenic determinants of a histocompatibility antigen (HLA-B7) induced by monoclonal antibodies. *J Immunol* 1984: **132**: 2975–83.
60. El-Awar N, Terasaki PI, Cai J et al. Epitopes of the HLA-A, B, C, DR, DQ and MICA antigens. *Clin Transpl* 2007: **175–94**.
61. Muller C, Ziegler A, Muller G, Schunter F, Wernet P. Monoclonal antibody (Tu48) defining alloantigenic class I determinants specific for HLA-Bw4 and HLA-Aw23,-Aw24 as well as -Aw32. *Hum Immunol* 1982: **5**: 269–81.
62. Marrari M, Conca R, Praticò-Barbato L, Amoroso A, Duquesnoy R. Brief report: why did two patients who type for HLA-B13 have antibodies that react with all Bw4 antigens except HLA-B13? *Transpl Immunol* 2011: **25**: 217–20.