



HLAMatchmaker-Based Analysis of Human Monoclonal Antibody Reactivity Demonstrates the Importance of an Additional Contact Site for Specific Recognition of Triplet-Defined Epitopes

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ABSTRACT: Five HLA-A3 reactive human monoclonal antibodies (mAbs) originating from a parous woman were screened against HLA-typed panels by means of complement-dependent lymphocytotoxicity, high-definition ELISA, and flow cytometry with single antigen beads. Antibody reactivity profiles were compared with triplet amino acid sequence polymorphisms identified by HLA-Matchmaker, and a three-dimensional structural modeling program (Cn3D of the National Center for Biotechnology Information) was used to determine the topography of epitopes recognized by each mAb. These mAbs originated from a woman who during pregnancy developed antibodies to the paternal HLA-A3 antigen of her child. Each mAb was specific for one mismatched triplet on HLA-A3, and the reactivity patterns of these IgM-type mAbs were practically the same in lymphocytotoxicity and antigen-binding assays. One mAb was specific for 163dT, a unique triplet present only on A3. The other mAbs reacted with 62Qe, 142mI, or 144tKr; these triplets are present on different groups of HLA-A alleles, some of which, however, did not react. Topographic modeling of triplet-defined epitopes identified clusters of polymorphic surface residues that were shared between reactive alleles. These clusters may serve as primary contact sites for the specificity-determining

complementarity-determining region (CDR) loops of antibody. The reactivity with these mAbs required also the presence of self-sequence elsewhere on the HLA molecular surface as a critical secondary contact site for antibody, likely through another CDR loop. For instance, the reactivity of the 62Qe-specific mAb required the presence of a glycine residue in position 56 and the reactivity of the 142mI-specific mAb required the presence of the GTLRG sequence in positions 79–83. Conversely, there were many other amino acid differences between the mAb-reactive alleles and HLA-A3 that did not prevent antibody binding. For instance, the 62Qe-specific mAb-reactive alleles had 35 and the 142mI-reactive alleles had 50 of such “permissive” residue differences. An HLA-Matchmaker-based analysis of the reactivity of human mAbs will increase our understanding of the structural definition of HLA epitopes and their reactivity with alloantibodies. *Human Immunology* 66, 749–761 (2005). © American Society for Histocompatibility and Immunogenetics, 2005. Published by Elsevier Inc.

KEYWORDS: HLA; HLA-Matchmaker; triplet; epitope; antibody

ABBREVIATIONS

AR average reactivity
CDC complement-dependent cytotoxicity
CDR complementarity-determining region
FCB flow cytometric binding
HDE high-definition ELISA

mAb monoclonal antibody
MW molecular weight
OD optical density
PPR percentage of positive reactions
RS reactivity score

INTRODUCTION

Class I HLA-specific alloantibodies play an important role in humoral rejection of organ transplants and im-

mune refractoriness of thrombocytopenic patients to random donor platelet transfusions. Serum testing with HLA-typed panels permits the determination of antibod-

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ies that are specific for so-called private and public epitopes on HLA antigens. Such information is clinically important for the identification of donors with acceptable HLA antigen mismatches.

Elucidation of the three-dimensional structure and amino acid sequence polymorphisms has increased our understanding of how HLA epitopes are recognized by antibodies [1–16]. This has permitted the development of an algorithm to determine donor-recipient HLA compatibility at the structural level. HLAMatchmaker considers triplets or sequences of three amino acids as key elements of immunogenic epitopes that can elicit specific alloantibodies [17, 18]. Each HLA antigen is considered a string of polymorphic triplets in antibody-accessible sequence positions; there are 132 polymorphic triplets in 40 sequence positions of the serologically defined HLA-A, -B, and -C antigens. Interestingly, most DNA-defined subtypes of HLA antigens involve variations within this triplet repertoire. This program determines donor-recipient HLA compatibility by identifying mismatched triplets through intralocus and interlocus comparisons in each polymorphic sequence position [17]. Studies of two large kidney transplant databases have indicated that grafts from donors with HLA-A, -B antigen mismatches but compatible at the triplet level have similar graft survival rates as the zero-HLA-A, -B antigen mismatches [19]. Matching at the triplet level is also beneficial in platelet transfusions [20] and corneal transplantation [21]. HLAMatchmaker has been implemented in finding donors with acceptable mismatches for highly sensitized kidney transplant patients [22–24].

Most studies on epitope structure have been performed with alloantibodies and mouse monoclonal antibodies (mAbs). This report describes serologic studies on human mAbs for which the HLA types of immunizer and antibody producer are known so that the antibody specificity patterns can be analyzed in relation to the immunizing mismatched triplets. This analysis considered the premise that the complementarity-determining regions (CDRs) of an antibody make contact with many sites of an antigen [25, 26]. Three CDRs are on the heavy chains of the antibody molecule, and three CDRs are on the light chains. The third CDR of the heavy chain primarily conveys the antibody specificity and the remaining CDRs participate in the complex formation between antibody and antigen. Three-dimensional structures of different antigen-antibody complexes have demonstrated that most CDRs of an antibody make contact with protein antigen [27–29]. The total contact area between antibody and antigen is ~ 700 to 850 \AA^2 [30–32]. An HLA molecule has a similarly sized surface area [33], so we can expect that the binding between HLA antigens and specific alloantibodies will involve a substantial portion of the HLA molecular surface.

Generally, an epitope on a protein antigen contains a few critical residues (hence our use of triplets) that provide dominant contributions to the binding energy with the specificity-conveying CDR3 loop [27]. Human mAbs reacting with multiple alleles with different amino acid sequences provide opportunities to investigate the epitope structure and to investigate which residues play a critical role in antigen-antibody binding. We describe here an HLAMatchmaker-based method to analyze antibody reactivity patterns with HLA-typed panels and how the results provide an insight into the structural basis of antibody-defined HLA epitopes.

MATERIALS AND METHODS

Human mAbs

This study addresses the reactivity patterns of five human mAbs generated as previously described [34–36]. They have the following local identification and 13th International Histocompatibility Workshop numbers: OK4F9 (W008), OK2F3 (W038), OK5A3 (W042), OK2H12 (W063), and OK4F10 (W064). All have the IgM, κ isotype except OK5A3, which has the IgM, λ isotype and were derived from a single bleed of a uniparous woman 70 days after delivery. Complement-dependent cytotoxicity (CDC) against a small informative lymphocyte panel was used throughout the selection procedure of Epstein-Barr virus transformants, and hybridomas were rigorously subcloned before supernatants were produced for and tested for antibody reactivity.

Complement-Dependent Lymphocytotoxic Antibody Screening

These mAbs were screened by standard CDC methods in nine laboratories that participated in Project 6 of the 13th International Histocompatibility Workshop. A total of 817 HLA-typed panel cells were used and most of them had been tested by DNA methods at the allelic level. CDC reactivity was scored according to the commonly used scale: 8 (strongly positive), 6 (positive), 4 (weakly positive), 2 (doubtful positive), and 1 (negative). For each mAb, we calculated the percentage of positive reactions (PPR) of panel cells expressing a given HLA allele and a reactivity score (RS) was determined by averaging the reactivity scores for that allele.

In this large-scale project, some panel cells in participating laboratories can be expected to exhibit aberrant reactivity and might affect the analysis of the CDC data. We have therefore used the following approach to identify panel cells with acceptable reactivity. For each panel cell, we calculated an average reactivity (AR) with all 198 mAbs in Sets 1 and 2 in Project 6. For the total panel of 817 cells, the AR had a mean value of 3.25 ± 0.62 (range: 1.67–6.16). One can also expect that the

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FIGURE 1 Determination of the HLA-A3 triplotype for the HLA-A2,A68; B7,B27; Cw2,Cw7 phenotype of the antibody producer. Mismatched triplets are shown in boxes.

A2	9F	16Gr	45rMe	56G	62Ge	66rKv	70aHs	74H	76Vd	80gTl	82IRg	90A	105S	107W	127K	142mT	144tKh
A68	9Y	16Gr	45rMe	56G	62Rn	66rNv	70aQs	74D	76Vd	80gTl	82IRg	90A	105S	107G	127K	142mT	144tKh
B7	9Y	16Gr	45Ee	56G	62Rn	66qly	70aQa	74D	76Es	80rNI	82IRg	90A	105P	107G	127N	142tI	144tQr
B27	9H	16Gr	45Ee	56G	62Re	66qlc	70aKa	74D	76Ed	80rTl	82ILr	90A	105P	107G	127N	142tI	144tQr
Cw2	9Y	16Sr	45Ge	56G	62Re	66qKy	70rQa	74D	76Vn	80rKI	82IRg	90A	105P	107G	127N	142tI	144tQr
Cw7	9D	16Gr	45Ge	56G	62Re	66qNy	70rQa	74AD	76Vs	80rNI	82IRg	90D	105P	107G	127N	142tI	144tQr
A3	9F	16Gr	45rMe	56G	62Qe	66rNv	70aQs	74D	76Vd	80gTl	82IRg	90A	105S	107G	127N	142mI	144tKr
A2	147W	149aAh	151aHv	156L	158A	163T	166Ew	171Y	177Ei	180Q	183A	186K	193Av	199A	207S	246A	253Q
A68	147W	149aAh	151aHv	156W	158A	163T	166Ew	171Y	177Ei	180Q	183A	186K	193Av	199A	207S	246vA	253Q
B7	147W	149aAr	151aRe	156R	158A	163E	166Ew	171Y	177Dk	180E	183P	186K	193Pi	199A	207G	246A	253E
B27	147W	149aAr	151aRv	156L	158A	163E	166Ew	171Y	177Ei	180Q	183P	186K	193Pi	199A	207G	246A	253E
Cw2	147W	149aAr	151aRe	156W	158A	163E	166Ew	171Y	177Ei	180Q	183Eh	186K	193Pv	199A	207G	246A	253E
Cw7	147L	149aAr	151aRa	156L	158A	163T	166Ew	171Y	177Ei	180Q	183Ep	186K	193Pi	199A	207G	246A	253Q
A3	147W	149aAh	151aHe	156L	158A	163dT	166Ew	171Y	177Ei	180Q	183P	186K	193Pi	199A	207G	246A	253E

AR of a panel cell is influenced by the number of antigens on that cell and better yet, the triplet repertoire. Therefore, we calculated an AR/T ratio for each panel cell by dividing the average AR with the number of different triplets on that panel cell as determined by HLAMatchmaker. For this panel of 817 cells, the average AR/T ratio was 0.070 (range: 0.036–0.146). There were 29 (or 3.5% of the panel) “overreactive” cells with AR > 4.00 and AR/T > 0.090, and there were 26 (or 3.2%) “underreactive” panel cells with AR < 2.50 and AR/T < 0.050; all of them were excluded from analysis. The remaining 760 panel cells were used to analyze CDC reactivity patterns.

Complement-Independent Antibody Testing

We used two antigen-binding methods. Because these mAbs had the IgM isotype, we used a FITC-conjugated F(ab')₂ goat anti-IgM reagent (Jackson Immunochemicals, West Groove, PA) as the second antibody. Flow cytometric binding (FCB) was performed with HLA antigen-coated beads (FlowPRA Single Antigen—Class I, One Lambda, Canoga Park, CA) with a FACScan (Becton Dickinson, Immunocytometry Systems, San Jose, CA) [37]. This study focused on HLA-A polymorphisms, and flow cytometric reagents were available for all HLA-A antigens except A36, A43, and A80. Reactions were scored according to previously described procedures [37] as strongly positive (++), positive (+), weakly or questionably positive (-/+) and negative (neg).

Antigen binding was also determined with ELISAs with single HLA antigens (Lambda Antigen Tray High Definition Class I; One Lambda). The protocol of the high-definition ELISA (HDE) test followed manufacturer’s instructions, and reactions were recorded as optical density (OD) values measured with an ELX 800 ELISA reader (Bio-Tek Instruments)

Triplotype Determination

These mAbs originated from a Caucasian woman who typed as HLA-A2,A68; B7,B27; Cw2,Cw7. She had developed broadly reactive anti-HLA antibodies (90% panel-reactive antibodies [PRA]) during pregnancy with

a child who typed as HLA-A2, -A3; B7, B70; Cw2, Cw7. This series of mAbs reacted with HLA-A3, and Figure 1 demonstrates the repertoire of self-triplets in the antibody producer and the mismatched triplets of the immunizing HLA-A3 (presumably the common A*0301 allele) are shown in boxes. They constitute the following triplotype: 62Qe, 142mI, 144tKr, 151aHe, 163dT.

Analysis of Antibody Reactivity Patterns With HLA-Typed Panels

The reactivity patterns of mAbs were analyzed with an HLAMatchmaker-based program (available at <https://tpis.upmc.edu>). The program identifies the mismatched triplets for each HLA antigen in the panel. Nonreactive HLA antigens may have mismatched triplets that are not recognized by antibody, and the program can remove these triplets from the other HLA antigens in the panel. The remaining mismatched triplets on reactive antigens are considered as potential targets for antibody.

In this study, we analyzed the reactions of each allele that shared one or more triplets with the immunizing HLA-A3. The analysis of the CDC data required at least four informative panel cells. There were 67 panel cells with alleles that lacked the triplets of the HLA-A3 triplotype. These cells gave uniformly negative reactions with the mAbs.

Statistical Analysis

Specificity analysis of mAb reactivity with HLA-typed panels was assessed with the χ^2 test with Yates correction by the STATISTICA software program (StatSoft, Tulsa, OK).

Structural Topography of Triplet Polymorphisms

No x-ray crystallography structural information is currently available for HLA-A3 and the other HLA molecules reactive with the mAbs in this study. Therefore, three-dimensional modeling for these molecules was performed by submitting HLA amino acid sequences retrieved from IMGT/HLA online database [38] to the GenO3D online molecular modeling server [39]. This Web-based service (<http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>) generates three-dimensional

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TABLE 1 Reactivity of OK2F3 is specific for HLA-A3

	Lymphocytotoxicity			Antigen binding	
	n	Positive reaction (%)	Reactivity score	Flow beads	High-definition ELISA ^a
HLA-A3	127	98	7.6	++	1270
Non-HLA-A3	633	1	1.1	Neg	189

^a Positive control, 1271; negative control, 222.

structural models of HLA chains without $\beta 2$ microglobulin or any peptide in the peptide-binding groove. Locations of triplets and polymorphic amino acid residues on HLA molecules were visualized with Vector NTI-3D Molecular Viewer software (Invitrogen Life Science Software, Frederick, MD, USA). This software can also measure distances between different residues and this information is useful for estimating CDR contact sites on HLA molecules. The antibody contact area of protein antigen is $\sim 700\text{--}850\text{ \AA}^2$ and involves generally five or all six CDRs [30–32]. A circular area of that size has a radius of $\sim 15\text{--}18\text{ \AA}$ and the specificity-determining CDR is often in a central location [26–29]. This means that the other CDRs can only contact residues that are less than 20 \AA away.

RESULTS

The specificity of each mAb correlated with one triplet of the immunizing HLA-A3 triplotype, namely 62Qe, 142mI, 144tKr, or 163dT. No other triplet was recognized. The reactivity patterns were practically identical in CDC and FCB assays. The HDE patterns were also consistent although as discussed below, some antigen-coated wells (especially A1) had low reactivity. These findings indicate that these mAbs had virtually identical reactivity patterns in complement-dependent and complement-independent assays. This was not surprising because these mAbs had all IgM isotypes, and it is well known that IgM-type antibodies fix complement very well.

Reactivity of Anti-A3-Specific mAb OK2F3

OK2F3 (W038) reacted only with the immunizing A3 by all techniques (Table 1). By CDC, the PPR was 98% and the RS was 7.6 for 127 A3-positive cells; these values were only 1% and 1.1 for 633 A3-negative cells. This mAb was also monospecific for A3 in FCB and HDE assays. Because A3 antigens carry a unique triplet, namely 163dT, it seems likely that this triplet is a specificity recognition site for one of the CDR loops of OK2F3.

Reactivity of Anti-62Qe-Specific mAb OK2H12 (W063)

The CDC reactivity pattern of OK2H12 exhibited the strongest correlation with the presence of 62Qe in the cell panel ($\chi^2 = 344.2$; $p < 10^{-8}$). This mAb reacted with A3 and exhibited high PPR and RS values with the 62Qe-carrying alleles A*0101, A*0103, A*1101, A*3201, A*3601, and A*7401 (Table 2). On the other hand, the 62Qe-carrying A*3001, A*3002, and A*3101 alleles gave negative reactions. The FCB reactivity pattern was consistent with the CDC data. The HDE assays gave practically the same results, although the OD values of A*0101 wells were rather low. OK2H12 was also tested on the LAT 1288 ELISA trays (One Lambda) with full HLA phenotypes, and the OD of four informative A1-containing wells was 476 ± 136 , and this value was considered weakly positive. A previous study with an ELISA kit from a different manufacturer has demonstrated high reactivity of HLA-A1 with OK2H12 [36]. In this study, the low ELISA reactivity of A*0101 may have a technical reason regarding the antigen preparation in the reagent kit. A*0101 beads reacted strongly in the FCB assays (Table 2).

These findings demonstrate that 62Qe defines the epitope recognized by OK2H12. This triplet plays a central role as the binding site for OK2H12, presumably by contacting one of the CDRs of this mAb. Topographic analysis identified two additional polymorphic residues that might participate as contact sites for OK2H12. All 62Qe-carrying alleles share 65R and 66N, and these polymorphic residues are located on the molecular surface in close proximity to 62Qe. It is therefore possible that the combination of 62Q, 63E, 65R, and 66N comprises the 62Qe-defined epitope making contact with the specificity-determining CDR loop of OK2H12.

But why does OK2H12 not react with A*3001, A*3002, and A*3101, which have the same 62Qe-defined epitope? These alleles must have a critical structural difference from A*0301 and the other reactive 62Qe-carrying alleles. A comparative analysis of the remaining 52 polymorphic positions in HLA-A sequences indicated position 56 as the only one that distinguished

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TABLE 2 Reactivity of OK2H12 is specific for 62Qe

Allele	Triplets shared with HLA-A3	n	Lymphocytotoxicity		Antigen binding	
			Positive reaction (%)	Reactivity score	Flow beads	High-definition ELISA ^a
A*0301	62Qe, 142mI, 144tKr, 151aHe, 163dT	103	99	7.6	++	1428
A*0101	62Qe, 142mI, 144tKr	61	100	7.6	**	159 ^b
A*0103	62Qe, 142mI, 144tKr	4	75	6.3	nt ^c	nt
A*1101	62Qe, 142mI, 144tKr	83	96	7.7	++	1131
A*3201	62Qe, 142mI	29	100	7.8	++	1256
A*3601	62Qe, 142mI, 144tKr	6	83	6.5	nt	459
A*7401	62Qe, 142mI	8	100	7.5	nt	1270
A*3001	62Qe, 142mI	12	0	1.0	Neg	52
A*3002	62Qe, 142mI	16	0	1.0	nt	nt
A*3101	62Qe, 142mI	38	8	1.6	Neg	53
A*2301	142mI	31	0	1.0	Neg	54
A*2402	142mI	107	4	1.2	Neg	nt
A*2403	142mI, 144tKr	4	0	1.0	nt	nt
A*2407	142mI, 144tKr	6	0	1.0	nt	nt
A*2501	142mI, 151aHe	11	0	1.0	Neg	108
A*2601	142mI, 151aHe	36	6	1.3	Neg	54
A*2901	142mI	24	4	1.2	Neg	109
A*3301	142mI	15	0	1.0	Neg	92
A*3303	142mI	16	0	1.0	nt	69
A*3401	142mI, 151aHe	22	3	1.2	nt	46
A*3402	142mI, 151aHe	11	9	1.7	Neg	nt
A*6601	142mI, 151aHe	14	0	1.0	Neg	43
A*8001	142mI, 144tKr	6	0	1.0	Neg	51
A2/A28	none	67	1	1.1	Neg	78

^a Positive control 1677; negative control, 56.
^b OD = 476 ± 136 for four informative A1-containing wells on LAT™ 1288 ELISA trays.
^c nt = not tested.

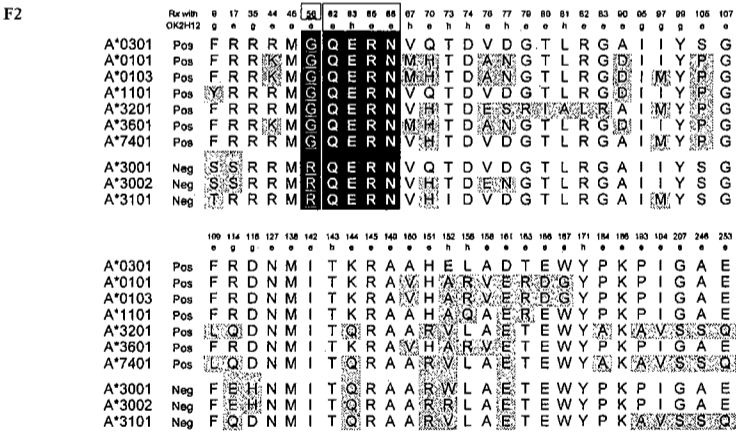
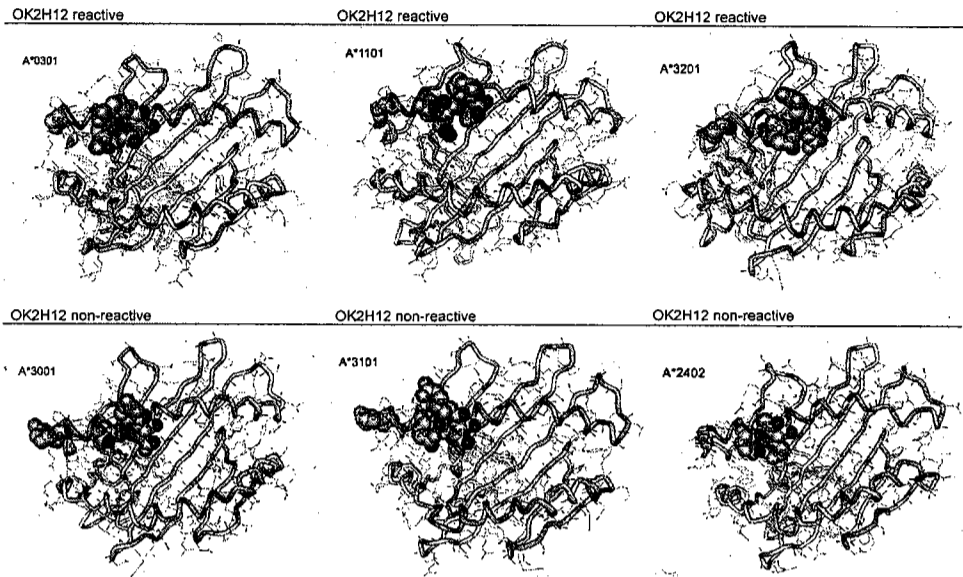


FIGURE 2 Polymorphic amino acid sequences of 62Qe-containing alleles reacting with OK2H12. The residue positions in A*0301 are exposed (e), hidden (h), or in the β-pleated sheets of the peptide-binding groove (g). The 62Qe-defined epitope comprises 62Q, 63E, 65R, and 66N (white type in black boxes). There is one consistent difference in position 56 (G vs. R, in gray boxes) between positively and negatively reacting alleles. Permissive substitutions are shown in light gray boxes.

these groups of 62Qe-carrying alleles (Figure 2). All OK2H12-reactive alleles have 56G (glycine), and the nonreactive alleles have 56R (arginine). These residues are very different from each other: glycine is a small (molecular weight [MW] = 57), nonpolar amino acid, whereas arginine is large (MW = 156) and positively charged. There are also marked differences in solvent accessibility, and we propose that the substitution G→R in position 56 will adversely affect the binding of OK2H12 to 62Qe-carrying alleles. 56G is a self-triplet expressed by antigens of the antibody producer (Figure 1) and we conclude that this triplet may represent a critical binding site for OK2H12, perhaps through contact with another CDR loop.

Figure 3 illustrates the topography of the 56G and the 62Qe-defined epitope (62Q, 63E, 65R, and 66N) on the three-dimensional structural model of A*0301 and two other OK2H12-reactive alleles, A*1101 and A*3201. They are ~11 Å apart; this distance seems sufficient to permit contacting by two different CDRs of antibody. The OK2H12-nonreactive A*3001 and A*3101 have a

FIGURE 3 Topography of polymorphic amino acid residues critical for binding to the 62Qe-specific mAb OK2H12. In the upper panels, three reactive alleles A*0301, A*1101, and A*3201 have the cluster of 62Q, 63E, 65R, and 66N that describes the 62Qe-defined epitope and 56G as a critical contact site. The lower panels show two non-reactive 62Qe-carrying alleles A*3001 and A*3101, which have 56R and the nonreactive A*2402 with a different cluster 62E, 63E, 65G, and 66K.



protruding arginine residue in position 56, whereas the 62Qe-defined epitope is not markedly changed. Figure 3 illustrates the nonreactive A*2402 that has 56G but a different amino acid combination: 62E, 63E, 65G, and 66K. In conclusion, OK2H12 is specific for a 62Qe-defined epitope, and its reactivity seems to require the presence of a self-sequence defined by 56G.

The HLA-A3 and the other OK2H12-reactive alleles have 35 residue differences in 32 sequence positions; 20 of them are antibody accessible, and 12 are below the molecular surface and in the β -pleated sheets. They are marked by gray boxes in Figure 2 and considered to be “permissive” substitutions because they did not prohibit the OK2H12 reactivity of 62Qe-carrying alleles. The 62Qe-carrying, OK2H12-nonreactive A*3001 and A*3002 alleles have uniquely different residues not present on HLA-A3, namely 17S (antibody accessible), 9S, 114E, and 116H (in the β -pleated sheets), and 152W and 152R (antibody inaccessible). On the other hand, the OK2H12 nonreactive A*3101 allele has no or permissive substitutions in these positions, except for a unique 9T. Because these residue differences do not exhibit any uniform pattern among A*3001, A*3002, and A*3101, we conclude that none of them represents major contributing factors to the OK2H12 nonreactivity of these alleles. It seems more likely that the substitution of the surface residue 56G by 56R is responsible for the lack of reactivity with this mAb.

Reactivity of Anti-142mI-Specific mAbs OK4F9 (W008) and OK4F10 (W064)

These mAbs demonstrated practically identical reactivity patterns in all assays (Table 3). They reacted with A3, and their CDC reactivity patterns exhibited the strongest

correlation with the presence of 142mI in the cell panel (OK4F9: $\chi^2 = 88.1, p < 10^{-8}$; OK4F10: $\chi^2 = 77.6, p < 10^{-8}$). The 142mI triplet had a high frequency (91%) in the CDC panel because it is present on all HLA antigens except A2, A68, and A69. OK4F9 and OK4F10 exhibited high PPR and RS values for 142mI-carrying alleles A*0101, A*1101, A*2601, A*2902, A*3001, A*3002, A*3101, A*3301, A*3303, A*3401, A*3402, A*6601, and A*7401. The FCB data revealed practically identical reactivity patterns as the CDC data. The HDE assays yielded also very similar results. Table 3 does not list A*3601 and A*8001 cells because fewer than four informative cells were available; almost all of them reacted in CDC.

Altogether, these findings indicate that OK4F9 and OK4F10 are specific for 142mI, which comprises an isoleucine in position 142 and the A locus-specific methionine in position 138. Although triplets generally consist of three contiguous amino acids, molecular modeling has demonstrated that 138M and 142I are right next to each other in antibody-accessible positions. Topographic analysis revealed that all 142mI-carrying alleles share two nearby polymorphic residues on the molecular surface, namely 143T and 145R, and it is conceivable that these residues contribute to the 142mI-defined epitope. Thus, the combination 138M, 142I, 143T, and 145R may serve as a contact site for the specificity-determining CDR loop of OK4F9 and OK4F10.

These mAbs did not react with 142mI-carrying A*2301, A*2402, A*2403, A*2407, A*2501, and A*3201. Considering the concept that these alleles lack a critical contact site for antibody, we compared their polymorphic residues with those of reactive 142mI-

T3 These mAbs demonstrated practically identical reactivity patterns in all assays (Table 3). They reacted with A3, and their CDC reactivity patterns exhibited the strongest

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TABLE 3 Reactivity of OK4F9 and OK4F10 with 142mI-carrying alleles^a

Allele	Triplets shared with HLA-A3	Lymphocytotoxicity					Antigen binding			
		Positive reaction (%)			Reactivity score		Flow beads		High-definition ELISA	
		n	OK4F9	OK4F10	OK4F9	OK4F10	OK4F9	OK4F10	OK4F9	OK4F10
A*0301	62Qe, 142mI, 144tKr, 151aHe, 163dT	79	99	84	7.6	6.6	++	++	1554	1596
A*0101	62Qe, 142mI, 144tKr	48	100	83	7.4	5.8	++	+	513	459
A*1101	62Qe, 142mI, 144tKr	68	97	93	7.8	7.3	++	++	1136	1299
A*2601	142mI, 151aHe	37	97	86	7.5	7.1	++	++	767	906
A*2902	142mI	16	100	94	8.0	7.6	++	++	1663	1642
A*3001	62Qe, 142mI	13	100	100	8.0	8.0	++	++	851	1025
A*3002	62Qe, 142mI	12	92	67	6.5	4.6	nt	nt	nt	nt
A*3101	62Qe, 142mI	29	97	86	7.6	6.9	++	++	755	780
A*3301	142mI	13	92	85	7.3	6.5	nt	nt	522	423
A*3303	142mI	12	75	75	6.3	6.3	++	++	1523	1597
A*3401	142mI, 151aHe	19	100	100	8.0	7.6	nt	nt	nt	nt
A*3402	142mI, 151aHe	7	100	100	8.0	7.4	++	+	1442	1672
A*6601	142mI, 151aHe	8	100	100	8.0	7.6	nt	+	1518	1620
A*7401	62Qe, 142mI	5	100	80	8.0	6.8	nt	+	1311	1272
A*2301	142mI	17	0	0	1.0	1.0	Neg	Neg	96	88
A*2402	142mI, 144tKr	78	8	8	1.6	1.5	Neg	Neg	76	49
A*2403	142mI, 144tKr	4	25	0	1.7	1.0	nt	nt	103	57
A*2407	142mI, 144tKr	5	0	0	1.0	1.0	nt	nt	nt	nt
A*2501	142mI, 151aHe	9	0	0	1.0	1.0	Neg	Neg	171	94
A*3201	62Qe, 142mI	22	0	0	1.0	1.0	Neg	Neg	153	68
A2/A28	none	67	6	3	1.3	1.2	Neg	Neg	117	72

OK4F9: positive control, 1436: negative control, 125. OK4F10: positive control, 1935; negative control, 107. nt = not tested.

F4 carrying alleles (Figure 4). A linear sequence of five polymorphic residues could be distinguished: The immunizing HLA-A3 and the reactive 142mI-carrying alleles have 79G-80T-81L-82R-83G, which is a self-sequence because it is also present in the A2 and A68 antigens of the antibody producer. The 138M, 142I, 143T, and 145R residues are clustered in the α2 domain, and the 79–83 sequence resides in the α1 domain (Figure 5). The distance between 142I and 82R is ~13 Å; this is far enough for contact by two separate CDRs of antibody. In contrast, the nonreactive alleles have the Bw4-like 79R-80I-81A-82L-83R sequence that might have been responsible for preventing binding. As illustrated in Figure 5, the nonreactive A*2402 and A*3201 have two large protruding 79R and 83R residues, and the 138M residue points toward the 79–83 sequence.

F5 These findings suggest that OK4F9 and OK4F10 are specific for a 142mI-defined epitope that presumably makes contact with the specificity-determining CDR loop, but their binding to antigen also requires contact of the self-sequence 79G-80T-81L-82R-83G through another CDR loop. HLA-A3 and the other reactive 142mI-carrying alleles have 50 residue differences in 38 of 45 remain-

ing polymorphic positions, including 26 that are antibody accessible and 13 are below the molecular surface and in the β-pleated sheets (gray boxes in Figure 4). They are considered permissive substitutions because they did not adversely affect the reactivity with OK4F9 and OK4F10.

Certain negatively reacting alleles have distinct polymorphic residue differences with HLA-A3 and the reactive alleles. Especially A*2301, A*2402, A*2403, and A*2407 have uniquely different 66G and 127K in antibody-accessible positions and 95L, 99F, 114H, and 116Y in the β-pleated sheet (Figure 4). Although these differences might have contributed to the nonreactivity of these alleles, they are absent on the nonreactive A*2501 and A*3201 alleles, which have no or permissive substitutions in these positions. All negative alleles have a unique 76E. This surface residue is in close proximity to the 79R-80I-81A-82L-83R sequence that we propose is responsible for the lack of binding of 142mI-carrying alleles to OK4F9 and OK4F10.

Reactivity of Anti-144tKr-Specific mAb OK5A3

Table 4 demonstrates that OK5A3 (W042) reacted in τ_4 CDC with immunizing A*0301 and the 144tKr-

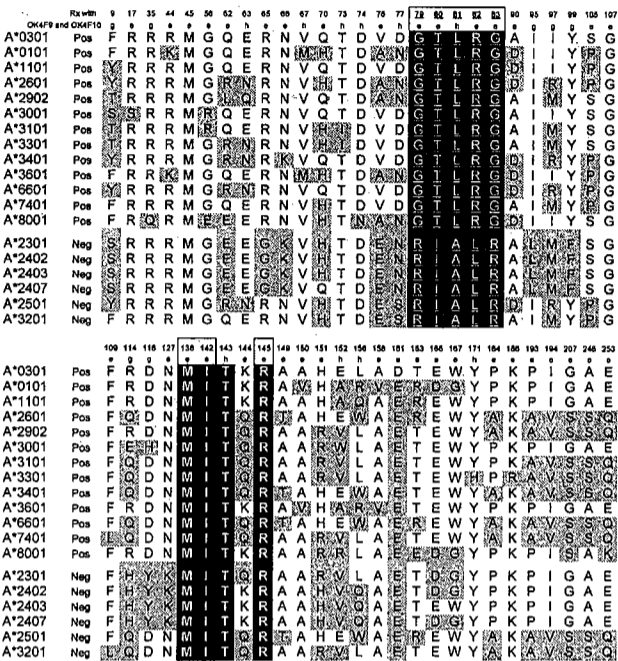


FIGURE 4 Polymorphic amino acid sequences of 142mI-containing alleles reacting with OK4F9 and OK4F10. The residue positions in A*0301 are exposed (e), hidden (h), or in the β -pleated sheets of the peptide-binding groove (g). The 142mI-defined epitope comprises 138M, 142I, 143T, and 145R (white type in black boxes). The positively and negatively reacting alleles exhibit a consistent difference in sequence positions 79–83 (shown in gray boxes). Permissive substitutions are shown in light gray boxes.

carrying alleles A*0101, A*1101, A*1102, A*2402, A*2403, A*2407, and A*3601. The CDC reactivity of this mAb had the strongest correlation with the presence of 144tKr in the cell panel ($\chi^2 = 397.9$, $p < 10^{-8}$). The FCB and HDE assays yielded practically the same reactivity patterns (Table 4), although in HDE, the A*0101 and A*3601 wells gave rather low OD values. This weak ELISA reactivity seemed to reflect a technical problem with these antigen preparations in this kit.

Sequence analysis demonstrates that the immunizing HLA-A3 and the other 144tKr-carrying, OK5A3-reactive alleles shared the nearby polymorphic residues 142I and 149A. Both of them are antibody accessible (Figure 6). Thus, the 144tKr-defined epitope may have the combination of 142I, 143T, 144K, 145R, and 149A as a possible contact site for the specificity-determining CDR3 loop of OK5A3.

The CDC panel had five informative 144tKr-carrying, A*8001-positive cells, but none of them reacted with OK5A3. No FCB data were available, but A*8001 was also nonreactive in HDE. Although OK5A3 was obviously specific for an epitope defined by 144tKr, the lack of reactivity with the 144tKr-carrying A*8001 indicated

that the structure of this antigen was critically different from A3 and the other reactive antigens. It is well known that A*8001 is, sequencewise, very different from the other HLA-A alleles [40]. Not surprisingly, a comparative analysis between A*8001 and the reactive 144tKr-carrying alleles demonstrated unique residue differences in six positions: 35, 56, 74, 151, 207, and 253 (Figure 6). HLA-A3 and the OK5A3-reactive alleles have 35R, 56G, 74D, 151H, 207G, and 253E; all of them are self-residues present in HLA antigens of the antibody producer.

A topographic analysis was performed to identify residues that might be potentially important as contact sites for antibody (Figure 7). The best candidates are 74D and 151H, as illustrated on A*0301 and A*2402. Each residue is $\sim 15 \text{ \AA}$ away from 144tKr epitope, sufficiently close for contact by different CDRs. The nonreactive A*8001 has a protruding 151R residue that might have interfered with OK3A3 binding. Similarly, A*8001 has an exposed 74N rather than the largely hidden 74D present on A*0301. A*8001 has a protruding 56E, which is $\sim 42 \text{ \AA}$ from the 144tKr; this distance seems too great to serve as a direct contact site for another CDR of OK5A3. Similarly, the distances between 144tKr and the $\alpha 3$ domain residues 207S and 253K are 43 \AA and 53 \AA , respectively, and it seems unlikely that these positions represent direct contact sites for the CDRs of OK5A3. Finally, A*8001 has a unique 35Q in the β -pleated sheet, but this involves an antibody-inaccessible position. Because A*8001 has so many structural differences with A*0301, it is possible that any combination might have prevented antibody binding.

OK5A3-reactive alleles had 34 residues in 30 polymorphic positions that were different from the immunizing HLA-A3, and 18 of them were antibody accessible (Figure 6). These residues represent permissive substitutions because they did not affect OK5A3 reactivity.

DISCUSSION

This report presents further evidence that HLAMatchmaker is a useful tool to analyze the structural basis of HLA determinants reacting with alloantibodies. Human mAbs offer the advantage that they react with single epitopes in the context of allorecognition. Such epitopes can be defined by comparing the triplet repertoires of the immunizing HLA antigen and the HLA antigens of the antibody producer.

These studies have produced three general findings about antibody reactivity with HLA antigens. First, we demonstrated the close relationship between triplets and epitopes. For this antibody producer, the immunizing HLA-A3 antigen had five potentially immunogenic

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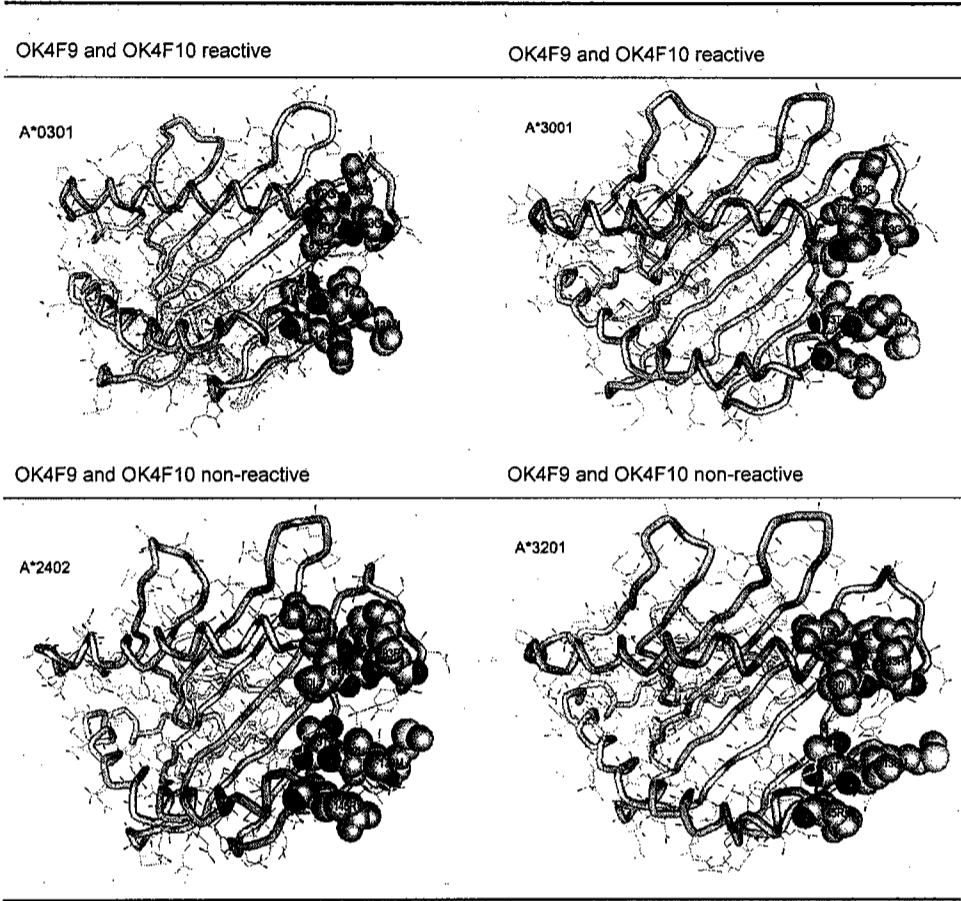


FIGURE 5 Topography of binding sites to the 142mI-specific mAbs OK4H9 and OK4F10. Both reactive A*0301 and A*3001 (upper panels) and nonreactive A*2402 and A*3201 (lower panels) have the cluster of 138M, 142I, 143T, and 145R, but they have different residues in positions 79–83, namely 79G, 80T, 81L, 82R, 83G versus 79R, 80I, 81A, 82L, 83R.

epitopes defined by mismatched triplets, and for four of them—62Qe, 142mI, 144tKr, and 163dT—we identified specific antibodies. This provides further support of the triplet-matching algorithm to determine class I HLA compatibility at the humoral immune level.

Specific antibody recognition is mediated by the CDR3 loop of the immunoglobulin heavy chain that makes contact with a short amino acid sequence of a protein antigen [28–33]. For the binding of HLA antigen to antibody, we propose that a polymorphic triplet constitutes a critical focus in contacting this CDR loop. Structural modeling permitted the identification of additional polymorphic residues that are in close proximity to the specific triplet and that are shared between antibody-reactive HLA alleles. For instance, all 62Qe-carrying alleles share 65R and 66N, and these nearby surface residues may also participate in the binding to the CDR3 loop of the 62Qe-specific mAb OK2H12. Similarly, the 142mI-specific OK4F9 and OK4F10 seem to have CDR3 loops that contact the combination of surface residues 138M, 142I, and 145R. OK5A3 is specific for 144tKr, and the reactive alleles also share 142I and 149A. Thus, the contact with the specificity-determining CDRs may involve more residues than the triplet

itself; this concept is consistent with observations on crystal structures of protein antigen-antibody complexes [28–33]. This notion does not necessarily change our concept that triplets can structurally define epitopes.

The second observation is that the triplet-specific antibody binding requires the presence of one or more additional residues shared between the immunizing antigen and the triplet-carrying reactive alleles. For instance, 62Qe-carrying alleles react with OK2H12 if they have a glycine residue in position 56; they are nonreactive if they have 56R. Similarly, the reactivity of the 142mI-specific OK4F9 and OK4F10 requires the presence of the GTLRG sequence in positions 79–83. This cluster is located closely enough (~10–15 Å) to serve as a critical contact site for another CDR, rather than the specificity-determining CDR loop itself. Interestingly, all are self-residues because they are present in the HLA antigens of the antibody producer.

This finding is similar to the data of Akkoc and Scornik [41] from absorption-elution studies on allosera that led to the conclusion that mismatched amino acid residues located in one part of the HLA molecule can interact with residues that are not different from those in the patient's own HLA molecules to form epitopes rec-

TABLE 4 Reactivity pattern of OK5A3 is specific for 144tKr

Allele	Triplets shared with HLA-A3	Lymphocytotoxicity			Antigen binding	
		<i>n</i>	Positive reaction (%)	Reactivity score	Flow beads	High-definition ELISA ^a
A*0301/2	62Qe, 142mI, 144tKr, 151aHe, 163dT	98	99	7.7	++	1706
A*0101	62Qe, 142mI, 144tKr	68	91	6.9	++	151
A*1101	62Qe, 142mI, 144tKr	74	97	7.8	++	1234
A*1102	62Qe, 142mI, 144tKr	4	100	7.0	nt ^b	nt
A*2402	142mI, 144tKr	129	95	7.6	nt	2243
A*2403	142mI, 144tKr	4	100	8.0	++	1910
A*2407	142mI, 144tKr	6	100	8.0	nt	nt
A*3601	62Qe, 142mI, 144tKr	7	100	7.7	++	216
A*8001	142mI, 144tKr	5	0	1.0	nt	69
A*2301	142mI	27	0	1.0	Neg	62
A*2501	142mI, 151aHe	12	0	1.0	Neg	63
A*2601/3	142mI, 151aHe	37	16	2.0	Neg	71
A*2901/2	142mI	22	5	1.3	Neg	90
A*3001	62Qe, 142mI	14	0	1.0	Neg	61
A*3002	62Qe, 142mI	17	0	1.0	Neg	nt
A*3101	62Qe, 142mI	26	15	2.1	Neg	49
A*3201	62Qe, 142mI	25	4	1.3	Neg	66
A*3301	142mI	11	9	1.6	Neg	101
A*3303	142mI	11	18	2.3	nt	93
A*3401	142mI, 151aHe	12	5	1.1	nt	62
A*3402	142mI, 151aHe	10	10	1.3	Neg	nt
A*6601/2	142mI, 151aHe	14	0	1.0	Neg	66
A*7401	62Qe, 142mI	9	0	1.1	nt	81
A2/A28	none	67	4	1.2	Neg	79

^a Positive control, 1901; negative control, 90.
^b nt = not tested.

ognizable by alloantibodies. Moreover, site-directed mutagenesis studies by Lutz and coworkers have identified additional residues that are critical for antibody binding to epitope-defining residues on HLA-B7 [11, 42]. For instance, the mouse mAb MB40.2 is specific for an epitope defined by residues in positions 177–180, but its binding requires also the presence of a glutamic acid residue in position 169. Mouse mAb BB7.1 is specific for an epitope in the 63–70 sequence but requires also residues 166E and 169R for binding [11].

The third observation is that mAb-reactive alleles have many residue differences with the immunizing HLA-A3. These differences are considered permissive substitutions because they do not affect antibody reactivity. They involve 32 sequence positions in 62Qe-carrying, OK2H12-reactive alleles, 38 positions in 142mI-carrying, OK4F9- and OK4F10-reactive alleles, and 30 positions in 144tKr-carrying, OK5A3-reactive alleles. Most of them are located in antibody-accessible positions, and many others are below the molecular surface including the β-pleated sheets. These findings are consistent with site-directed mutagenesis data of

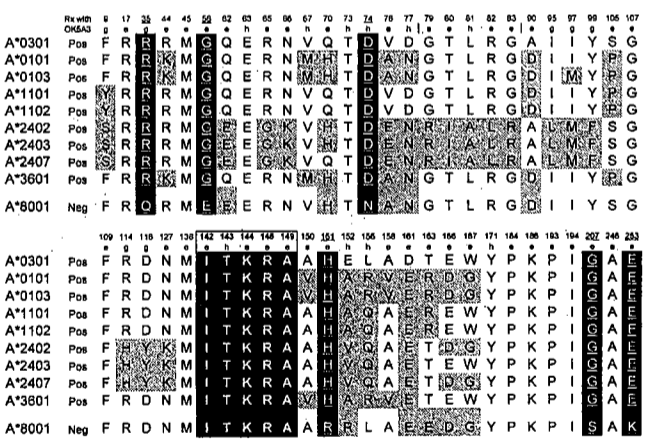


FIGURE 6 Polymorphic amino acid sequences of 144tKr-containing alleles reacting with OK5A3. The residue positions in A*0301 are exposed (e), hidden (h), or in the β-pleated sheets of the peptide-binding groove (g). The 144tKr-defined epitope comprises 142I, 143T, 144K, 145R, and 148A (white type in black boxes). The 144tKr-carrying A*8001 has residue differences with the positively reacting allele in six sequence positions 35, 56, 74, 151, 207, and 253 (shown in gray boxes). Permissive substitutions are shown in light gray boxes.

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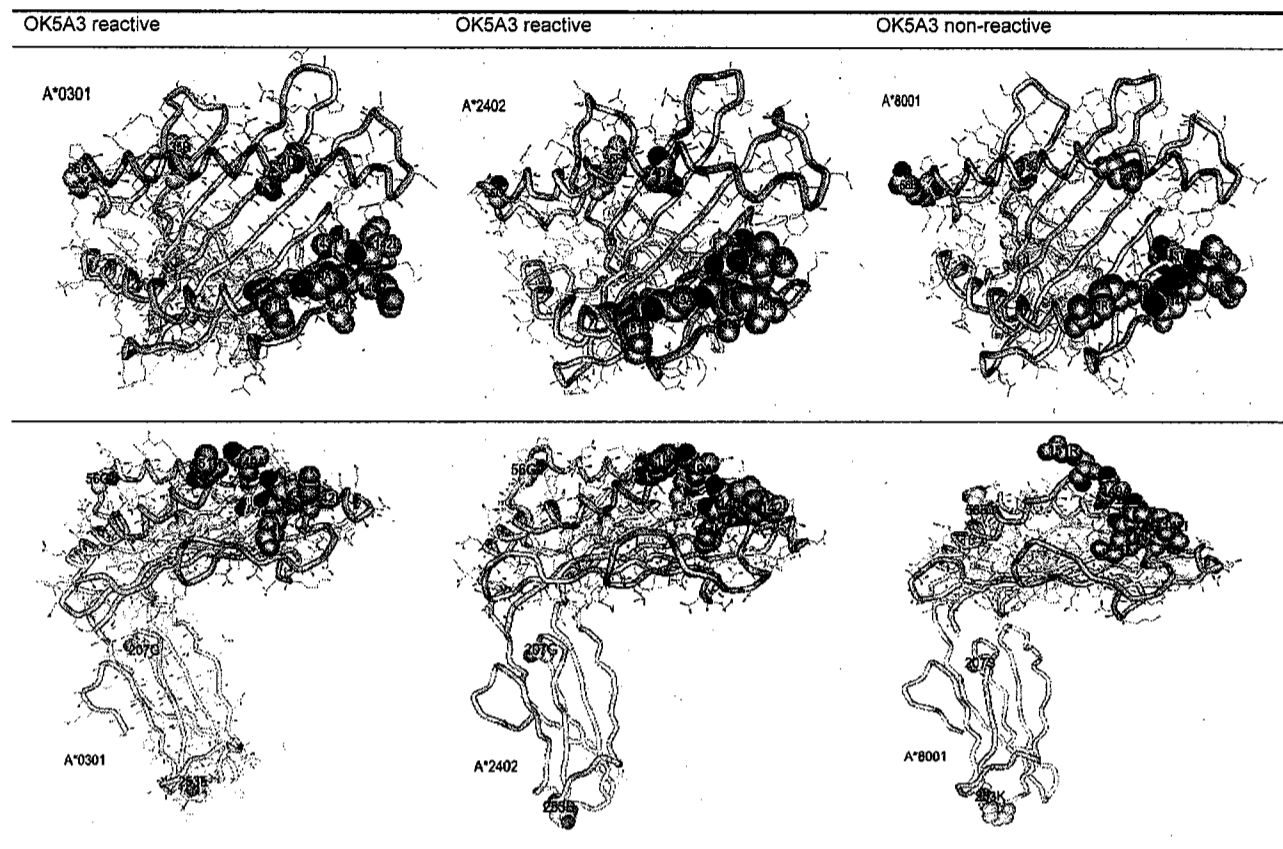


FIGURE 7 Topography of the OK5A3-reactive A*0301 and A*2402 and the nonreactive A*8001. The upper panels illustrate the locations of sequence positions 35, 56, 74, and 151 in relation to the location of the 144tKr-defined epitope comprised of 142I, 143T, 144K, 145R, and 149A. The lower panels are side views depicting positions 207 and 253 in the third domain. They also demonstrate how 151R protrudes from the surface of A*8001.

Smith and colleagues [42] that most amino acid residue substitutions outside the epitope area do not alter binding of specific antibodies. Most striking is the observation that none of 70 single-residue substitutions in 62 positions spread out over the HLA-B7 molecule diminished the binding of a 75R,79R,82R-defined Bw6-like epitope to the Bw6-specific mAb SFR8-B6 [43]. Interestingly, only one substitution—alanine by glutamic acid in position 90—abolished binding with SFR8-B6. The antibody-accessible 90A residue is ~10 Å away from 79R, and it seems likely that this residue can make contact with a CDR that is different from the specificity-determining CDR of SFR8-B6.

Altogether, these findings suggest that HLA-specific antibodies have one CDR that conveys specificity for a triplet-defined epitope, but that they must engage at least a second CDR that interacts with a sequence not far away in the HLA molecular structure. This concept is relevant to a better understanding of humoral sensitization, especially in relation to HLA immunogenicity and serologic cross-reactivity due to epitope sharing. Most highly sensitized patients have antibodies restricted to

one or few epitopes that can be defined with triplets on the immunizing antigens. A negative reaction of a given antigen is interpreted as evidence that patient's antibodies do not recognize any epitope on that antigen, and this information can be applied to identify additional antigens as acceptable mismatches. However, a negative reaction might also be due to the absence of a second amino acid configuration critical for antibody binding, although the antigen carries the putative epitope. An improper interpretation of a negative reaction will lead to an erroneous result. As an example, a highly sensitized patient may have an antibody against 142mI with similar reactivity as mAb OK4F9 (Table 3). The serum PRA would be more than 80%, but the negative reactions with 142mI-carrying A23, A24, A25, and A32 (which have a different sequence RIALR in positions 79–83) do not imply that 142mI is an acceptable triplet. On the other hand, unpublished observations have identified highly sensitized patients with antibodies that react with all 142mI-carrying antigens, and their reactivity does not appear to require the same 79–83 sequence. Other

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sensitized patients have antibodies that do not react with 142mI and for them, this triplet would be acceptable.

This type of analysis of human mAbs offers an attractive approach to determine the structural basis of HLA immunogenicity and antigenicity. A collaborative study is now being conducted under auspices of the 14th International HLA and Immunogenetics Workshop (<http://www.microbiol.unimelb.edu.au/micro/14ihiws/newsletter.html>).

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REFERENCES

1. Santos-Aguado J, Barbosa JA, Biro PA, Strominger JL: Molecular characterization of serologic recognition sites in the human HLA-A2 molecule. *J Immunol* 141:2811, 1988.

2. Park MS, Terasaki PI, Barbetti A, Han H, Cecka JM: Significance of the HLA molecular structure to transplantation. *Clin Transplant* 1988:301, 1988.

3. Muller CA, Engler-Blum G, Gekeler V, Steiert I, Weiss E, Schmidt H: Genetic and serological heterogeneity of the supertypic HLA-B locus specificities Bw4 and Bw6. *Immunogenetics* 30:200, 1989.

4. Harprecht K, Olde K, Westphal E, Muller-Ruchholtz W: Serological mapping of HLA-epitopes with monoclonal antibodies and its interpretation by sequenced HLA-molecules. *Tissue Antigens* 34:170, 1989.

5. Fuller AA, Rodey GE, Parham P, Fuller TC: Epitope map of the HLA-B7 CREG using affinity-purified human alloantibody probes. *Hum Immunol* 28:306, 1990.

6. Fuller AA, Trevithick JE, Rodey GE, Parham P, Fuller TC: Topographic map of the HLA-A2 CREG epitopes using human alloantibody probes. *Hum Immunol* 28: 284, 1990.

7. Park M, Clark B, Maruya E, Terasaki P: HLA class I epitopes accounted for by single residues. *Clin Transplant* 1991:335, 1991.

8. Hildebrand WH, Madrigal JA, Belich MP, Zemmour J, Ward FE, Williams RC, Parham P: Serologic cross-reactivities poorly reflect allelic relationships in the HLA-B12 and HLA-B21 groups: dominant epitopes of the alpha 2 helix. *J Immunol* 149:3563, 1992.

9. Hildebrand WH, Madrigal JA, Little AM, Parham P: HLA-Bw22: a family of molecules with identity to HLA-B7 in the alpha 1-helix. *J Immunol* 148:1155, 1992.

10. Madrigal JA, Hildebrand WH, Belich MP, Benjamin RJ, Little AM, Zemmour J, Ennis PD, Ward FE, Petzl-Erler ML, du Toit ED: Structural diversity in the HLA-A10 family of alleles: correlations with serology. *Tissue Antigens* 41:72, 1993.

11. McCutcheon JA, Lutz CT: Mutagenesis around residue 176 on HLA-B*0702 characterizes multiple distinct epitopes for anti-HLA antibodies. *Hum Immunol* 35:125, 1992.

12. McCutcheon JA, Smith KD, Valenzuela A, Aalbers K, Lutz CT: HLA-B*0702 antibody epitopes are affected indirectly by distant antigen residues. *Hum Immunol* 36:69, 1993.

13. Parham P, Arnett KL, Adams EJ, Barber LD, Domena JD, Stewart D, Hildebrand WH, Little AM: The HLA-B73 antigen has a most unusual structure that defines a second lineage of HLA-B alleles. *Tissue Antigens* 43:302, 1994.

14. Hildebrand WH, Domena JD, Shen SY, Lau M, Terasaki PI, Bunce M, Marsh SG, Guttridge MG, Bias WB, Parham P: HLA-B15: a widespread and diverse family of HLA-B alleles. *Tissue Antigens* 43:209, 1994.

15. Little AM, Domena JD, Hildebrand WH, Shen SY, Barber LD, Marsh SG, Bias WB, Parham P: HLA-B67: a member of the HLA-B16 family that expresses the ME1 epitope. *Tissue Antigens* 43:38, 1994.

16. Parham P, Adams EJ, Arnett KL: The origins of HLA-A,B,C polymorphism. *Immunol Rev* 143:141, 1995.

17. Duquesnoy RJ: HLAMatchmaker: A molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. *Hum Immunol* 63:339, 2002.

18. 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. *Hum Immunol* 63:353, 2002.

19. 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* 75:884, 2003.

20. Nambiar A, Duquesnoy RJ, Adams S, Oblitas J, Leitman S, Stroncek D, F M: HLAMatchmaker-driven analysis of response to HLA matched platelet transfusions in alloimmunized patients. *Blood* (in press), 2005.

21. Boehringer D, Reinhard T, Duquesnoy R, Boehringer S,

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Enczmann J, de Lange P, Claas F, Sundmacher R: Beneficial effect of matching at the HLA-A and B amino-acid triplet level on rejection free survival in penetrating keratoplasty. *Transplantation* 77:417, 2004.

22. Duquesnoy RJ, Howe J, Takemoto S: HLA-Matchmaker: a molecularly based algorithm for histocompatibility determination. IV. An alternative strategy to increase the number of compatible donors for highly sensitized patients. *Transplantation* 75:889, 2003.

23. Duquesnoy RJ, Witvliet MJ, Doxiadis IIN, de Fijter H, Claas FHJ: HLA-Matchmaker-based strategy to identify acceptable HLA class I mismatches for highly sensitized kidney transplant candidates. *Transplant Int* 7:31, 2004.

24. 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* 78:190, 2004.

25. Kabat EA, Wu TT, Bilofsky H: Attempts to locate residues in complementarity-determining regions of antibody-combining sites that make contact with antigen. *Proc Natl Acad Sci U S A* 73:617, 1976.

26. Kabat EA: The structural basis of antibody complementarity. *Adv Protein Chem* 32:1, 1978.

27. Getzoff ED, Tainer JA, Lerner RA, Geysen HM: The chemistry and mechanism of antibody binding to protein antigens. *Adv Immunol* 43:1, 1988.

28. MacCallum RM, Martin ACR, Thornton JM: Antibody-antigen interactions: contact analysis and binding site topography. *J Mol Biol* 262:732, 1996.

29. Davies D, Cohen G: Interactions of protein antigens with antibodies. *Proc Natl Acad Sci U S A* 93:7, 1996.

30. Colman PM: Structure of antibody-antigen complexes: implications for immune recognition. *Adv Immunol* 43: 99, 1988.

31. Laver WG, Air GM, Webster RG, Smith-Gill SJ: Epitopes on protein antigens: misconception and realities. *Cell* 61:553, 1990.

32. Van Regenmortel MHV: Molecular dissection of protein antigens. In Sela M (ed): *The Antigens*, 1992.

33. Saper M, Bjorkman P, Wiley DC: Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution. *J Mol Biol* 219:277, 1991.

34. 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* 42:27, 1993.

35. Mulder A, Kardol M, Niterink J, Parlevliet J, Marrari M, Tanke J, Bruning J, Duquesnoy R, Doxiadis I, Claas F: Successful strategy for the large scale development of HLA-specific human monoclonal antibodies. In Charron D (ed): *12th International Histocompatibility Workshop*, vol 2. Paris, EDR Publishers, Paris, France, 1996.

36. 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* 56:106, 1997.

37. 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* 75:43, 2003.

38. Robinson J, Waller M, Parham P, de Groot N, Bontrop R, Kennedy LJ, Stoehr P, Marsh SG: IMGT/HLA and IMGT/MHC: sequence databases for the study of the major histocompatibility complex. *Nucleic Acids Res* 31: 311, 2003.

39. Combet C, Jambon J, Deleage G, Geourjon C: Gen03D: automatic comparative molecular modeling of protein. *Bioinformatics* 18:213, 2002.

40. Domena JD, Hildebrand WH, Bias WB, Parham P: A sixth family of HLA-A alleles defined by HLA-A*8001. *Tissue Antigens* 42:156, 1993.

41. Akkoc N, Scornik JC: HLA epitope matching. Contribution of matched residues to epitopes recognized by alloantibodies. *Transplantation* 52:903, 1991.

42. 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. *Immunol Res* 16:243, 1997.

43. Lutz CT, Smith KD, Greazel NS, Mace BE, Jensen DA, McCutcheon JA, Goeken NE: Bw4-reactive and Bw6-reactive antibodies recognize multiple distinct HLA structures that partially overlap in the alpha-1 helix. *J Immunol* 153:4099, 1994.