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Structural aspects of human leukocyte antigen class I epitopes detected by human monoclonal antibodies

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ABSTRACT

This study addresses the concept that human leukocyte antigen (HLA) class I–specific alloantibodies are specific for epitopes that correspond to HLAMatchmaker-defined eplets. Eplets are essential parts of so-called structural epitopes that make contact with the 6 complementarity determining regions of an antibody. From published molecular models of crystallized protein antigen–antibody complexes, we have calculated that contact residues on structural HLA epitopes should reside within a 15-Å radius of a mismatched eplet. This study addresses the structural basis of high-frequency HLA class I epitopes reacting with human monoclonal antibodies (mAbs) derived from women sensitized during pregnancy. All mAbs were tested in Luminex assays with single HLA allele panels. The HLAMatchmaker algorithm was used to determine their specificity in context with eplet sharing between the immunizing allele and antibody-reactive alleles. To assess the autoreactive B cell origin of these antibodies, we have applied the recently developed nonself–self paradigm of epitope immunogenicity to analyze residue differences between the immunizer and the alleles of the antibody producer. A total of 9 mAbs were specific for epitopes associated with the 41T, 80NRG, 163LW, 69AA, or 80ERILR eplets. In each case, the immunizing allele had within 15 Å of the mismatched eplet, no residue differences with 1 of the alleles of the antibody producer. This observation is consistent with the concept that these mAbs originated from B cells with self HLA immunoglobulin receptors. Eplet-carrying alleles exhibited different levels of reactivity, which, when compared with the immunizing allele, ranged from high to intermediate to very low. In many cases, lower reactivities were associated with differences from self to nonself residues in surface locations within 15 Å of the specific eplet. Apparently, such locations may serve as critical contact sites for the antibody. In other cases, other residue differences did not appear to affect binding with the antibody, suggesting that these locations do not play a major role in antibody binding. For these mAbs we did not obtain convincing evidence that residue differences in hidden positions below the molecular surface had significant effects on antibody binding. These findings have increased our understanding of the structural basis of the immunogenicity and antigenicity of HLA class I epitopes and provide a basis for interpreting HLA antibody reactivity patterns in Luminex assays with single alleles.

1. Introduction

Human leukocyte antigen (HLA) antibodies are significant risk factors for transplant rejection and failure and it has become evident that such antibodies are specific for epitopes rather than antigens. Epitopes are important not only for identifying acceptable mismatches for sensitized patients but also for a better understanding of the sensitization process induced by an HLA mismatch. Certain epitopes are located on 1 or a few HLA antigens, whereas others are shared by large groups of HLA antigens. Antibodies to high-frequency epitopes are generally responsible for the broad serum reactivity in highly sensitized patients. With a few exceptions, such as the Bw4 and Bw6 epitopes, there is little structural information about high-frequency epitopes and how HLA mismatches elicit specific antibody responses.

The analysis of reactivity patterns of human monoclonal antibodies (mAbs) with single HLA class I allele panels offers an attractive approach to identify HLA epitopes. Detailed information about the HLA molecular structure and amino acid sequence differences between reactive and nonreactive alleles has provided a basis for a structural characterization of HLA epitopes.

Interpretations of mAb reactivity patterns should be based on current concepts about the molecular structure of the antigen–antibody interface. Each antibody makes contact with an antigen

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via the loops of 3 complementarity determining regions (CDRs) of the heavy and light chains [1,2]. The centrally located third loop of the heavy chain (CDR-H3) has by far the greatest sequence diversity and plays a dominant role in determining antibody specificity [3–5]. Stereochemical analyses of crystallized antigen–antibody complexes have defined a structural epitope as the part of the antigen that makes contact with the antibody [5–7]. A structural epitope has about 15 to 25 contact residues in a surface area of 700 to 900 Å^2. Within it lies the so-called functional epitope consisting of amino acid residues that play a major role in the specific binding with antibody [8–12]. To be immunogenic, a structural epitope must have a functional epitope with at least 1 antibody-accessible nonself residue.

These concepts have been applied to HLAMatchmaker, a theoretical algorithm that considers that each HLA antigen represents a collection of amino acid patches in antibody-accessible positions [13–15]. These so-called eplets represent key elements of functional epitopes that can elicit HLA-specific alloantibody responses. From a more or less circularly sized surface area of 700 to 900 Å^2, one can calculate which potential contact residues of the structural epitopes would be within a radius of about 15 Å from a centrally located eplet.

Certain antibodies are specific for single eplets, but many others recognize epitopes represented by pairs of eplets in different sequence locations far enough from each other for contact by separate CDRs of the antibody [16–18]. Our studies on human mAbs have demonstrated that epitopes defined by eplet pairs always involve 1 nonself eplet and a self eplet shared between the immunizing antigen and the antibody producer [16,18]. This suggests an autoreactive component of the alloantibody response to an HLA mismatch; this concept has been recently expanded [19].

During B cell development, V_H and V_L gene rearrangements produce a diversity of immunoglobulin (Ig) receptors that can recognize epitopes on autologous proteins [20,21]. Following positive and negative selection processes [22–26] and receptor editing [27–29], the remaining B cells carry only low-avidity Ig receptors for self proteins, likely including those specific for self HLA epitopes. Their interactions with self HLA would not lead to B cell activation, but exposure to nonself HLA epitopes can induce strong alloantibody responses. The so-called nonself–self paradigm of HLA epitope immunogenicity considers the activation of self HLA–specific B cells by a distinct nonself eplet, whereas the remainder of the structural epitope on the immunizing antigen must be virtually identical to a corresponding self HLA epitope of the antibody producer [19]. A recent analysis of 6 mAbs specific for eplet pairs [19] and another study of highly reactive sera from 2 transplant candidates [30] have provided experimental support of this paradigm. These findings suggest that HLA antibodies originate from B cells with self HLA Ig receptors that recognize mismatched eplets within structural epitopes on immunizing antigens.

Affinity maturation is an important component of the transformation of activated B lymphocytes into antibody-producing cells [31]. This process involves somatic hypermutations of rearranged V_H and V_L gene segments that lead to stronger antibody binding with the antigen. This generally affects a few CDR loops that interact with certain amino acid configurations on structural epitopes and the other residues have less dominant roles. For HLA epitopes defined by eplet pairs, the nonself eplet is considered a specific recognition site and the self eplet functions as a critical contact site for a separate CDR loop of antibody [16]. Other locations within structural epitopes seem to play a minor role and amino acid substitutions do not affect reactivity with the antibody.

Characterizations of mAb-defined epitopes can be performed in context with amino acid configurations of the immunizing antigen and reactive alleles in Luminex panels, as well as the HLA type of the antibody producer. Human mAbs are ideal agents for the experimental verification of HLA epitopes. Because of their monoclonality, each must react with a single epitope presented by an immunizing mismatched antigen and shared with antibody-reactive alleles. HLAMatchmaker comparisons of the HLA types of the immunizing antigen and antibody producer often identify multiple mismatched eplets, but only 1 of them contributes significantly to an epitope recognized by a given mAb. This report describes reactivity patterns of mAbs specific for high-frequency HLA class I epitopes. The application of HLAMatchmaker and the nonself–self paradigm of HLA epitope immunogenicity have permitted structural descriptions of these epitopes.

2. Subjects and methods

2.1. Human monoclonal antibodies

This study was performed using IgG- and IgM-type mAbs derived from Caucasoid women (Dutch) who became sensitized during pregnancy by paternal antigens [32–34]. All were supernatants of cloned hybridomas generated from Epstein–Barr virus–transformed B cells. Four-digit HLA types of antibody producers and immunizers were determined by standard serologic and molecular methods, either by direct typing or by assignments based on allele frequencies.

2.2. HLA antibody reactivity testing

Human mAbs were tested with microbead Luminex assays using single HLA class I allele kits from 2 commercial vendors: One Lambda, Inc. (OL; Canoga Park, CA) and Gen-Probe Corporation (GP; Stamford, CT) according to the manufacturer's instructions. In brief, an aliquot of a mixture of Luminex microspheres, each coated with a single antigen, was incubated with 30 μL (OL) or 10 μL mAb (GP) and washed to remove unbound antibody. Antihuman immunoglobulin (IgG or IgM) antibody conjugated to phycoerythrin was added; after incubation the bead mixture was diluted for analysis using a LABScan 100 instrument (Luminex, Austin, TX) and reactivity was determined with the manufacturer's software. Median fluorescence intensity (MFI) values were recorded for each allele and the positive and negative control beads. Positive control beads in the Luminex kits are IgG specific; hence, they are not available for IgM antibodies. All mAbs exhibited extremely low MFI values (mostly $<$50) with the self alleles of antibody producer. A given allele was considered mAb reactive if the MFI values were consistently high in comparison with the immunizing antigen and controls, including self alleles of the antibody producer. The Student *t* test was used to determine the significance of differences between MFI values of various allele groups.

2.3. HLAMatchmaker analysis

We have used HLAMatchmaker to determine which eplets are shared by mAb-reactive alleles in the panel. A Microsoft Excel program that analyzes antibody reactivity for eplets and eplet pairs can be downloaded (http://www.HLAMatchmaker.net). There are worksheets to enter HLA types of the antibody producer, immunizer, and the allele panel, as well as the MFI values that can be readily copied from the manufacturer's Luminex database. HLAMatchmaker automatically calculates the average MFI of the antibody producer's self alleles and with this information one can determine and enter the cutoff MFI value for negative reactions. Negative alleles have eplets and eplet pairs that do not react with antibody and HLAMatchmaker automatically removes these epitopes from the panel. The reactive alleles show what eplets and eplet pairs are shared with the immunizer. We have used the allele frequency database (http://bioinformatics.nmdp.org/HLA/Haplotype_Frequencies/) to calculate epitope frequencies in North American Caucasians [35].

2.4. Nonself–self paradigm of epitope immunogenicity

This paradigm is based on the concept that HLA antibodies originate from B cells with Ig receptors for structural self HLA epitopes. Structural epitopes have surface areas of 700 to 900 Å^2 and one can calculate that antibody-contacting residues would be within a radius of 15 Å from a centrally located eplet. The Cn3D structure and sequence alignment software program [36] can visualize the locations of eplet residues on crystallographic structures of HLA molecules downloaded from http://www.ncbi.nlm.nih.gov/Structure. This program has a "select by distance" command that has been used to determine which residues are located within a radius of 15 Å of each eplet. Such residues are in monomorphic and polymorphic positions, and with HLA structural models we can determine which residues are visible on the molecular surface or in antibody-inaccessible hidden positions. We have developed an Excel program for the nonself–self paradigm to determine polymorphic residue differences within a 15-Å radius of an eplet on the immunizer and the alleles of the antibody producer. The goal is to identify the antibody producer's structural epitope that has an identical or very similar residue composition as the immunizing epitope with the mismatched eplet.

3. Results

Each mAb analysis consists of 4 steps. First, we determine eplets that are shared between the immunizing allele and all mAb-reactive alleles in the Luminex panels. This permits the assignment of the epitope specificity of the mAb. Conversely, all remaining alleles, including self-alleles of the antibody producer, must have extremely low MFI values because they lack the specific epitope. Second, with the Cn3D program we determine with informative molecular models the locations of specific eplets and surface residues within 15 Å that might serve as potential contact sites for the antibody. Illustrations of top views and side views of these molecular configurations provide some assessment of potential dimensions of structural epitopes. Monomorphic residues are depicted by sequence numbers, whereas relevant polymorphic residues also give amino acid descriptions with the standard letter code. The third step addresses the validity of the nonself–self paradigm of HLA epitope immunogenicity by comparing surface-exposed polymorphic residues within a 15-Å radius of the specific eplet between the immunizer and the alleles of the antibody producer. Accordingly, at least 1 allele should have no or very few residue differences with the immunizing allele. The final step considers the phenomenon of affinity maturation, whereby because of Ig gene mutations, some of the CDRs bind with increased strengths to certain residue configurations on structural epitopes [31]. Some residues would function as critical contact sites for the antibody, whereas residues in other locations do not seem to affect antibody binding. Accordingly, we have determined residue differences between mAb-reactive alleles in the Luminex panels and which ones are associated with decreased antibody binding.

3.1. ROU9A6 is specific for an epitope defined by 41T

The producer of ROU9A6 was typed as HLA-A*01:01, A*25:01; B*08:01, B*18:01; C*07:01, −. This mAb was specific for 41T shared by the immunizing B*44:03 and all strongly reactive alleles in the Luminex panels (Table 1). The self alleles and all other 41T-negative alleles had very low MFI values. The HLA-B eplet has a frequency of 48%. Figure 1a illustrates the location of 41T on the B*44:03 molecular structure and 2 views of surface residues within 15 Å, the presumed structural epitope for 41T. It can be readily seen that the centrally located 41T protrudes from the molecular surface. The immunizing B*44:03 has 6 polymorphic surface residues within 15 Å of 41T, namely 43P, 44R, 46E, 65Q, 69T, and 71T (Table 1). The antibody producer's B*08:01 and B*18:01 have 41A plus exactly the same residues within 15 Å as B*44:03, but the other alleles have multiple residue differences (A*01:01 = 5, A*25:01 = 4, and C*07:01 = 2). This suggests that the structural epitope of the immunizing B*44:03 has the nonself 41T plus a self configuration present in one of the antibody producer's alleles, namely B*08:01 or B*18:01. Therefore, ROU9A6 appears to originate from a B cell with an Ig receptor for self 41A, plus this self configuration, and that the mismatched 41T triggered the activation and subsequent production of this antibody.

Table 1
Reactivity of ROU9A6 (IgG) with a 41T-defined epitope

		Eplet:	41T	Polymorphic surface residues within 15 Å of 41T						
		Sequence positions	41	43	44	46	65	69	71	
		Immunizing allele								
		B*44:03	T	P	R	E	Q	T	T	Number of differences
		Antibody producer								
		A*01:01	A	Q	K	–	R	A	S	5
		A*25:01	A	Q	–	–	R	A	S	4
		B*08:01	A	–	–	–	–	–	–	0
		B*18:01	A	–	–	–	–	–	–	0
		C*07:01	A	–	–	–	–	R	A	2
OL MFI	GP MFI	Panel								
9488	15847	Positive control								
9	87	Negative control								
15174	nt	B*13:01	T	–	–	A	–	–	–	
19404	18245	B*13:02	T	–	–	A	–	–	–	
10610	22974	B*40:01	T	–	–	–	–	–	–	
18522	21031	B*40:02	T	–	–	–	–	–	–	
16626	nt	B*40:06	T	–	–	–	–	–	–	
20733	21957	B*41:01	T	–	–	–	–	–	–	
18295	7464	B*44:02	T	–	–	–	–	–	–	
18305	14695	B*44:03	T	–	–	–	–	–	–	
21450	19061	B*45:01	T	–	–	–	–	–	–	
17208	20014	B*47:01	T	–	–	–	–	–	–	
13830	15192	B*49:01	T	–	–	–	–	–	–	
17934	12216	B*50:01	T	–	–	–	–	–	–	
15 ± 10	69 ± 12	Self alleles								
76 ± 233	81 ± 41	Other 41T-negative alleles								

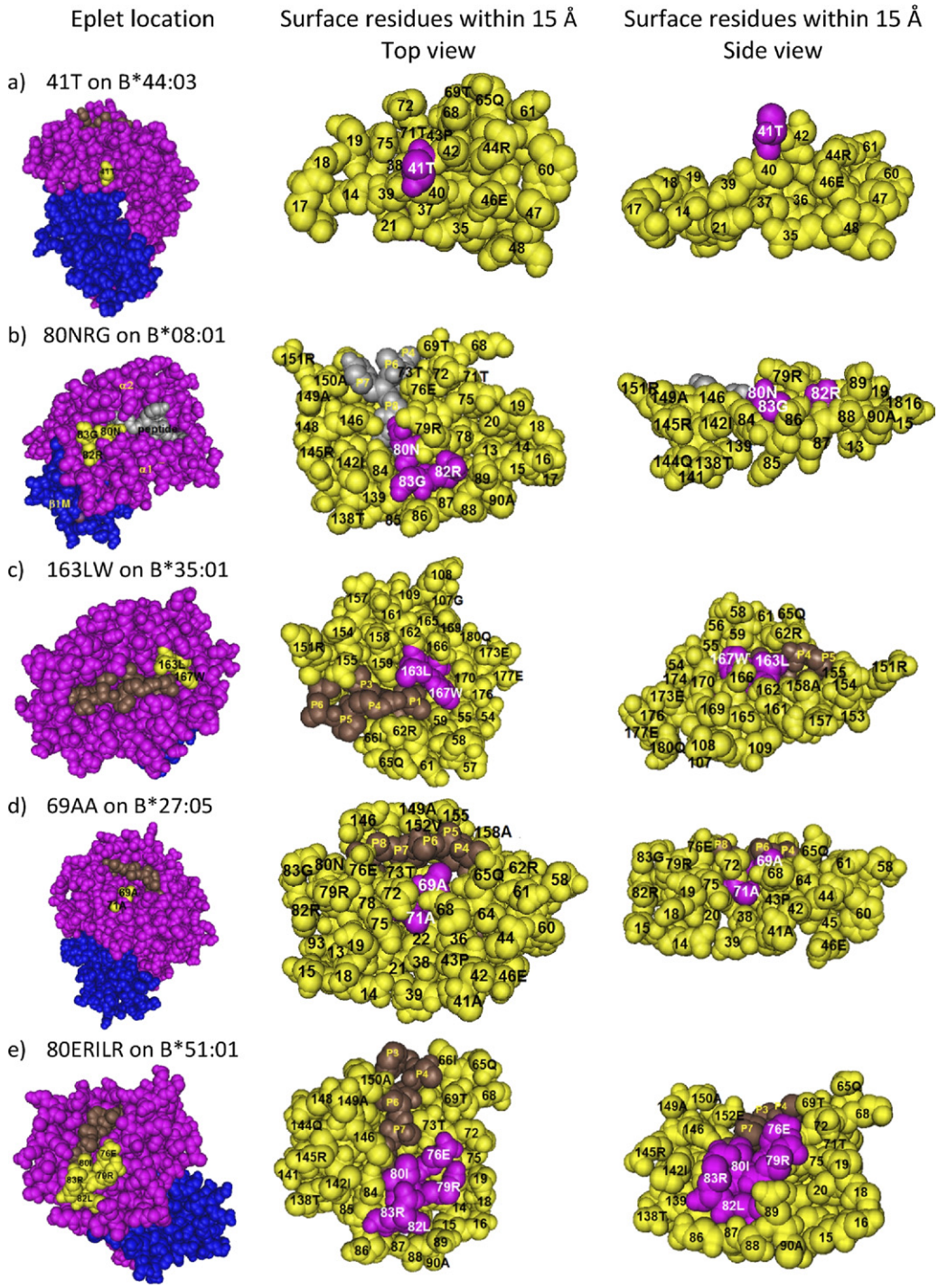

Fig. 1. Molecular models of representative crystalline HLA structures showing eplet locations (left column) and two views of surface-exposed residues within a 15 Ångstrom radius. Single numbers refer to sequence locations of monomorphic residues and numbers with letters represent locations with residue polymorphisms shared between the immunizing allele and the alleles of the antibody producer and the Luminex panel.

In both OL and GP Luminex panels, all ROU9A6-reactive alleles have the same residue compositions within the 15-Å radius of 41T as the immunizing B*44:03, except B*13:01 and B*13:02, which have 46A rather than 46E (Table 1). This substitution in sequence position 46 did not significantly affect antibody binding.

3.2. The 80NRG eplet shared by HLA-B and HLA-C alleles

Two mAbs were specific for 80NRG. The B*07:02-induced KAM3H9 (IgM) reacted with 80NRG-carrying HLA-B and HLA-C alleles (Table 2). This eplet is defined by 80N, 82R, and 83G and has a frequency of 89% on HLA-B and 87% on HLA-C alleles. Figure 1b shows the location of 80NRG on a molecular model of B*08:01 (none was available for B*07:02) and 2 views of surface residues within 15 Å. The centrally located 80NRG eplet appears to lie flat on the molecular surface and is surrounded by residues on the α1 and α2 helices, as well as some peptide residues. The immunizing B*07:02 has 11 polymorphic surface residues within 15 Å of 80NRG and they are identical for the antibody producer's B*27:05, which has 80TLR (Table 2). Other alleles have residue differences ranging from 2 for B*37:01 to 8 for A*02:01. Thus, KAM3H9

Table 2
Reactivity of KAM3H9 (IgM) with 80NRG on HLA-B and HLA-C alleles

		Eplet	80NRG			Polymorphic surface residues within 15 Å of 80NRG											
		Sequence positions:	80	82	83	69	71	73	76	79	90	138	142	144	145	151	
		Immunizing allele															Number of differences
		B*07:02	N	R	G	A	A	T	E	R	A	T	I	Q	R	R	
		Antibody producer															
		A*02:01	T	R	G	–	S	–	V	G	–	M	T	K	H	H	8
		B*27:05	T	L	R	–	–	–	–	–	–	–	–	–	–	–	0
		B*37:01	T	L	R	T	T	–	–	–	–	–	–	–	–	–	2
		C*02:02	K	R	G	R	–	–	V	–	–	–	–	–	–	–	2
		C*06:02	K	R	G	R	–	A	V	–	D	–	–	–	–	–	4
OL MFI	GP MFI	Panel															
nt	nt	Positive control															
3	9	Negative control															
11198	12614	B*07:02	N	R	G	–	–	–	–	–	–	–	–	–	–	–	
10529	12460	B*54:01	N	R	G	–	–	–	–	–	–	–	–	–	–	–	
9731	13270	B*55:01	N	R	G	–	–	–	–	–	–	–	–	–	–	–	
10505	12283	B*56:01	N	R	G	–	–	–	–	–	–	–	–	–	–	–	
13359	nt	B*67:01	N	R	G	–	–	–	–	–	–	–	–	–	–	–	
13714	12826	B*27:08	N	R	G	–	–	–	–	–	–	–	–	–	–	–	
13143	12983	B*42:01	N	R	G	–	–	–	–	–	–	–	–	–	–	–	
13838	11977	B*81:01	N	R	G	–	–	–	–	–	–	–	–	–	–	–	
12309	nt	B*82:01	N	R	G	–	–	–	–	–	–	–	–	–	–	–	
nt	12730	B*82:02	N	R	G	–	–	–	–	–	–	–	–	–	–	–	
nt	4897	B*07:03	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
12887	11516	B*08:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
10326	nt	B*14:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
8687	nt	B*14:02	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
nt	5722	B*14:05	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
nt	6095	B*14:06	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
12789	12499	B*15:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
13353	12381	B*15:02	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
12632	12080	B*15:03	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
10698	nt	B*15:10	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
11858	nt	B*15:11	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
13071	12524	B*15:12	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
nt	10147	B*15:18	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
13420	12593	B*18:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
13664	11553	B*35:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
nt	12407	B*35:08	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
11589	12059	B*39:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
7473	11123	B*40:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
10877	12418	B*40:02	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
9864	nt	B*40:06	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
12380	9641	B*41:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
12475	11142	B*45:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
11598	7559	B*48:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
11299	7840	B*50:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
12261	11442	B*78:01	N	R	G	T	T	–	–	–	–	–	–	–	–	–	
10682	3975	B*73:01	N	R	G	–	–	–	V	–	D	–	–	–	–	–	
10585	4951	B*46:01	N	R	G	R	–	–	V	–	–	–	–	–	–	–	
9042	5616	C*01:02	N	R	G	R	–	–	V	–	–	–	–	–	–	–	
9146	nt	C*03:02	N	R	G	R	–	–	V	–	–	–	–	–	–	–	
9713	7689	C*03:03	N	R	G	R	–	–	V	–	–	–	–	–	–	–	
12024	8531	C*03:04	N	R	G	R	–	–	V	–	–	–	–	–	–	–	
nt	5272	C*07:01	N	R	G	R	–	A	V	–	D	–	–	–	–	–	
8880	5021	C*07:02	N	R	G	R	–	A	V	–	D	–	–	–	–	–	
6918	9345	C*08:01	N	R	G	R	–	–	V	–	–	–	–	–	–	–	
nt	8966	C*08:02	N	R	G	R	–	–	V	–	–	K	–	–	–	–	
nt	10781	C*12:02	N	R	G	R	–	A	V	–	–	–	–	–	–	–	
6939	nt	C*12:03	N	R	G	R	–	A	V	–	–	–	–	–	–	–	
8652	8680	C*14:02	N	R	G	R	–	–	V	–	–	–	–	–	–	–	
7068	1046	C*16:01	N	R	G	R	–	–	V	–	–	–	–	–	–	–	
347 ± 283	10 ± 4	Self alleles															
188 ± 262	12 ± 5	Other 80NRG-negative alleles															

appears to originate from an autoreactive B cell specific for self 80TLR with a 15-Å^2 configuration on B*27:05 and the nonself 80NRG on the immunizing B*07:02 provided the trigger of activation leading to antibody production.

In these Luminex panels, all HLA-B alleles with 80NRG reacted well with KAM3H9, and 10 alleles (B*27:08, B*42:01, etc.) have identical residue compositions as the immunizing B*07:02. A rather large group of alleles (B*07:03, B*08:01, etc.) have residue differences in 2 positions, from 69A to 69T (69A → T) and 71A → T, but they did not appear to affect binding with KAM4A9. Similarly, the 90D residue difference with B*73:01 had no significant effect. B*46:01 and the 80NRG-carrying HLA-C alleles had slightly but significantly lower MFI values than the other 80NRG-carrying HLA-B alleles (OL, 8,896 ± 1,651 vs 11,743 ± 1,574, $p < 0.001$; GP, 6,899 ± 2,787 vs 10,784 ± 2,675, $p < 0.001$). These alleles have residue differences in a few surface positions, including the 69A → R and 76E → V substitutions, which appear to have only a modest effect on binding with KAM3H9.

Table 3
Summary of the reactivity of 80NRG-specific OUW4F11 (IgG)

Immunizing allele	Number of residue differences		
	B*08:01	or	C*07:01
Antibody producer			
A*03:01	9		9
A*68:02	11		11
B*27:05	2		5
B*44:03	0		6
C*02:02	4		3
Luminex panel	OL MFI		GP MFI
Positive control	9,024		10,760
Negative control	7		300
80NRG-positive HLA-B alleles except B46	12,826 ± 3,270*		9,588 ± 4,605**
80NRG-positive HLA-C alleles plus B46	9,028 ± 1,965*		10,975 ± 4,672**
Self alleles	13 ± 8		257 ± 24
Other 80NRG-negative alleles	13 ± 6		281 ± 49

*$p < 0.001$.
**$p > 0.5$ (not significant).

OUW4F11 is the second 80NRG-specific mAb (Table 3). There are 2 possibilities for the immunizing allele. B*08:01 is most likely because this allele has no residue differences within 15 Å^2 with the antibody producer's B*44:03, which has 80TLR. The other possibility is C*07:01, which has 3 differences with the antibody producer's C*02:02, which has 80KRG. Either case is consistent with the concept that OUW4F11 originated from a self HLA-reactive B cell. Luminex testing indicated somewhat lower MFI values for the 80NRG-positive HLA-C alleles in the OL kit, but the differences with HLA-B were not significant with the GP kit (Table 3).

3.3. Identification of 2 163LW-defined epitopes

VD1F11 (IgM) reacted with all alleles that shared 163LW defined by 163L and 167W (Table 4). This eplet is on 55% of HLA-B alleles and 29% of HLA-C alleles. The immunizing antigen is unknown, so we could not use the nonself–self paradigm for autoreactivity analysis. Figure 1c depicts the locations of 163LW and the surface residues within 15 Å on a B*35:01 molecular model. This eplet is embedded and 2 polymorphic residues 62R and 65Q appear to protrude from the molecular surface. VD1F11 gave generally moderately high MFI values with all 163LW-positive alleles (except B*52:01 in the GP kit). Its reactivity with self alleles was very low, but a few 163LW-negative HLA-A and HLA-C alleles exhibited very weak reactivity without any recognizable specificity pattern. Also, it is not understood why the 163LW-negative C*18:01 in the GP kit had an MFI of 4,185, whereas the surface residue identical C*18:02 in the OL kit had an MFI of only 223.

The B*15:03-induced OK6H10 (IgM) and OK6H12 (IgM) were also specific for 163LW (Table 4). All 9 polymorphic surface residues within 15 Å are also present on the antibody producer's B*27:05, which has 163EW. This suggests that OK6H10 and OK6H12 originated from B cells with Ig receptors for self 163EW on B*27:05 and that the mismatched 163LW must have been the activating stimulus that led to antibody production.

Both mAbs had similar reactivity patterns with the Luminex panels (Table 4). Most well-reacting 163LW-carrying alleles have identical polymorphic residues within 15 Å of 163LW as the immunizing B*15:03. Four alleles (B*15:16, B*57:01, B*57:03, and B*58:01) have 66N instead of 66I; their average MFI values were lower than those with 66I (OK6H10, OL, 3,316 vs 7,133, $p < 0.0001$; GP, 1,727 vs 4,611, $p < 0.03$; OK6H12, OL, 3,099 vs 6,893, $p < 0.0001$; GP, 1,712 vs 4,678, $p < 0.03$). Thus, the 66I → N substitution decreases binding with the mAbs; the same can be said about the 62R → G and 65Q → R differences for the B17 alleles. By contrast, B*46:01, C*03:02, C*03:03, and C*03:04 had extremely low MFI values; all of them have 66K rather than 66I, whereas the 3 Cw3 alleles differ also by 173K. These findings suggest that sequence position 66 serves as a critical contact site for OK6H10 and OK6H12; the 66I → K substitution almost abolishes the binding with antibody but 66I → N has only a modest effect.

The 163LW-carrying B*51:01, B*52:01, and B*78:01 have identical residue compositions within 15 Å as the immunizing B*15:03 but much lower MFI values with the GP kit than the OL kit (Table 4). These differences were reproducible with repeat testing. Because these alleles in the GP kit had high MFI values with other mAbs (data not shown), we have ruled out a technical problem, although we cannot offer an explanation for these discrepant reactions between both kits.

3.4. Two epitopes associated with 69AA

The 69AA eplet defined by 69A and 71A has a frequency of 45%. The B*55:01-induced MUL9E11 (IgG) and the B*07:02-induced VTM9A10 (IgG) were specific for 69AA but showed differences in their MFI values with the allele panels (Table 5). A nonself–self analysis indicated that the B*18:01 allele of the MUL9E11 producer has no residue differences within 15 Å of 69AA of the immunizing B*55:01 and B*15:01 of the VTM9A10 producer has the 46E → A difference with the immunizing B*07:02. Figure 1d shows the locations of 69AA and 46E (they are 13.5 Å apart) and the other residues within a 15 Å radius on B*27:05. These findings are consistent with the concept that both mAbs originated from autoreactive B cells with self HLA Ig receptors.

These mAbs had different reactivity patterns with 69AA-carrying alleles (Table 5). MUL9E11 gave high MFI values with most of them except the negatively reacting B*73:01, which has a 76V rather than 76E. This residue position is about 5.5 Å away from 69AA, a sufficient distance for contact with a second CDR besides the CDR specific for 69AA. The weakly reacting B*27:03 and B*27:05 have 80T rather than 80N; this residue difference is 11.5 Å away from 69AA, a sufficient distance for contact with a third CDR of antibody. It should be noted that the 80N → I as well as the 46E → A, 62R → G, 65Q → R, 66I → N, 82R → L, and 83G → R substitutions on B*15:16, B*57:01, B*57:03, and B*58:01 did not affect binding with MUL9E11. Thus, MUL9E11 appears to be specific for an epitope defined by 69AA in combination with self 76E and to a lesser extent with self 80N.

VTM9A10 had a different reactivity pattern with 69AA-carrying alleles (Table 5). MUL9E11 had low MFI values with B*73:01, B*27:03, and B*27:05 but these alleles reacted well with VTM9A10, although the MFI of B*73:01 in the GP was not very high. This suggests that the 76E → V and 80N → T substitutions in these alleles do not affect binding with VTM9A10. In contrast, B*15:16, B*57:01, B*57:03, and B*58:01 had very low MFI values, which were nevertheless higher than those with the 69AA-negative alleles, including those of the antibody producer. The weakly reacting 69AA-carrying alleles had distinct residue differences, namely 46E → A, 62R → G, 65Q → R, 66I → N, and 80N → I, which must have affected their binding with VTM9A10. The remaining residue differences between 69AA-carrying alleles in positions 59Y → H, 82R → L, 83G → R, and 158A → T had no effect. Thus, VTM9A10 appears to be specific for an epitope defined by 69AA in combination with the self eplet 62R65Q66I (about 5 Å away) and/or self 80N.

The findings suggest that the different reactivity patterns of the 69AA-specific MUL9E11 and VTM9A10 are associated with residue differences within the structural epitope defined by 69AA.

3.5. A Bw4-related epitope

The well-defined Bw4 epitope corresponds to 82LR and the A*24:02-induced MUS4H4 specifically recognizes this epitope on A23, A24, A25, A32, and all Bw4-carrying HLA-B antigens (Table 6). The 82LR eplet is on 30% of HLA-A and 62% of HLA-B alleles. In contrast, the B*49:01-induced KAL3D5 reacts well with all these alleles except B13, B27, B37, B44, and B47. The KAL3D5-reactive

Table 4
Reactivities of 3 mAbs with 163LW-defined epitopes

						Eplet	163LW		Polymorphic surface residues within 15 Å of 163LW									
						Sequence position	163	167	62	65	66	107	109	151	173	177	180	
Immunizing allele						Immunizing allele												
Unknown						B*15:03	L	W	R	Q	I	G	L	R	E	E	Q	Number of differences
Antibody producer						Antibody producer												
A*01:01						A*02:01	T	W	G	R	K	W	F	H	–	–	–	6
A*03:01						A*68:01	T	W	–	R	N	–	F	H	–	–	–	4
B*07:02						B*07:02	E	W	–	–	–	–	–	–	–	D	E	2
B*08:01						B*27:05	E	W	–	–	–	–	–	–	–	–	–	0
C*07:01						C*02:02	E	W	–	–	K	–	–	–	–	–	–	1
C*07:02						C*07:02	T	W	–	–	K	–	–	–	–	–	–	1
VD1F11 (IgM)		OK6H10 (IgM)		OK6H12 (IgM)														
OL MFI	GP MFI	OL MFI	GP MFI	OL MFI	GP MFI	Panel												
nt	nt	nt	nt	nt	nt	Positive control												
9	11	7	8	7	11	Negative control												
8362	9077	8273	6776	8125	6817	B*15:01	L	W	–	–	–	–	–	–	–	–	–	
7226	10319	9194	8053	8972	8233	B*15:02	L	W	–	–	–	–	–	–	–	–	–	
6474	5000	7803	10176	7638	9996	B*15:03	L	W	–	–	–	–	–	–	–	–	–	
8361	nt	8666	nt	8241	nt	B*15:10	L	W	–	–	–	–	–	–	–	–	–	
5416	5442	7498	2648	7300	2595	B*15:13	L	W	–	–	–	–	–	–	–	–	–	
nt	5672	nt	3990	nt	3960	B*15:18	L	W	–	–	–	–	–	–	–	–	–	
7244	11559	8811	7916	8625	8453	B*35:01	L	W	–	–	–	–	–	–	–	–	–	
nt	11837	nt	4435	nt	4599	B*35:08	L	W	–	–	–	–	–	–	–	–	–	
4577	7091	6758	2105	6438	2032	B*49:01	L	W	–	–	–	–	–	–	–	–	–	
7812	5087	9946	1494	9862	1451	B*50:01	L	W	–	–	–	–	–	–	–	–	–	
6010	6736	7491	8716	7292	9106	B*53:01	L	W	–	–	–	–	–	–	–	–	–	
5275	10565	6582	7268	6252	7251	B*56:01	L	W	–	–	–	–	–	–	–	–	–	
5586	2297	2105	16	1903	19	B*51:01	L	W	–	–	–	–	–	–	–	–	–	
7879	nt	8695	nt	8339	nt	B*51:02	L	W	–	–	–	–	–	–	–	–	–	
2049	235	4188	368	3899	407	B*52:01	L	W	–	–	–	–	–	–	–	–	–	
5547	7456	3847	597	3620	577	B*78:01	L	W	–	–	–	–	–	–	–	–	–	
3749	3655	3930	115	3702	115	B*57:01	L	W	G	R	N	–	–	–	–	–	–	
3749	nt	3428	nt	3087	nt	B*57:03	L	W	G	R	N	–	–	–	–	–	–	
2783	6992	3729	2944	3554	2837	B*58:01	L	W	G	R	N	–	–	–	–	–	–	
5480	12413	2179	2123	2055	2184	B*15:16	L	W	–	–	N	–	–	–	–	–	–	
5172	6652	112	28	111	33	B*46:01	L	W	–	–	K	–	–	–	–	–	–	
8056	nt	837	nt	796	nt	C*03:02	L	W	–	–	K	–	–	–	K	–	–	
7453	10588	451	494	438	488	C*03:03	L	W	–	–	K	–	–	–	K	–	–	
7532	11425	585	666	554	681	C*03:04	L	W	–	–	K	–	–	–	K	–	–	
19 ± 10	19 ± 13	10 ± 4	10 ± 3	10 ± 4	12 ± 3	Self alleles												
102 ± 262[a]	117 ± 521[b]	10 ± 4	12 ± 13	10 ± 5	11 ± 3	163LW-negative alleles												

[a]Includes A*11:02 = 416, A*66:01 = 1,299, C*06:02 = 566, C*08:01 = 1,127, C*12:03 = 1,195, and C*14:02 = 455.
[b]Includes A*66:01 = 426, C*08:01 = 589, C*12:02 = 974, C*14:02 = 549, and C*18:01 = 4,115.

alleles share the 80I residue, which has 76E, 79R, 82L, and 83R within 3 Å, suggesting that the eplet can be described as 80ERILR. This eplet has a frequency of 30% on HLA-A and 31% on HLA-B. The antibody producer's B*08:01 has within 15 Å of its self 80ERNRG no residue differences with the immunizing B*49:01, which supports the contention that KAL3D5 originated from an autoreactive B cell.

All 80ERILR-carrying alleles reacted equally well with KAL3D5, although there were multiple residue differences with the immunizing B*49:01: 43P → Q, 65Q → G, 66I → K or N, 69T → A, 71T → S or A, 90A → D, 138T → M, 144Q → K, and 149A → T (Table 6). They can be considered permissible substitutions because they do not affect the binding ability of the structural epitope recognized by KAL3D5. In contrast, the 80I → T substitution drastically reduced the binding with KAL3D5, including those 80T-carrying alleles, such as B*37:01 and B*44:02, that have identical residue compositions within 15 Å^2 as the immunizing B*49:01 (Table 6). Thus, 80I is the dominant component of the 80ERILR eplet recognized by a specific CDR of KAL3D5. Figure 1e shows the residue locations within 15 Å^2 of 80ERILR. Although there are many permissible substitutions indicated by informative alleles, it is possible that certain locations on the molecular surface might be contacted by other CDRs of KAL3D5. Current Luminex panels do not have informative alleles to address this issue.

It should be noted that MUS4H4 reacted well with both 80ERILR- and 80ERTLR-carrying alleles (Table 6). This antibody specifically recognizes 82LR rather than the 76E79R part of these configurations because 76E79R-carrying alleles with different residues in positions 80, 82, and 83 were always negative (data not shown).

3.6. Effect of hidden residues on epitope antigenicity

This analysis has also assessed the influence of residue differences in hidden, antibody-inaccessible positions within 15 Å of the eplet-defined epitopes. It is possible that some unexposed residues may alter the conformation of surface epitopes and affect their binding with antibody. For the 6 epitopes analyzed in this study, the corresponding alleles had a total of 90 hidden locations with residue differences with the immunizing alleles (Table 7). We observed no differences in 79 instances (88%). There were 11 distinct differences on less reactive alleles, including 9 on B*15:16, B*57:01, B*57:03, and B*58:01, which exhibited very low MFI values with the 69AA-specific VTM9A10, as indicated in Table 5. It should be pointed out that these very weakly reactive alleles also have residue differences in 7 antibody-accessible sequence positions. Similarly, B*46:01, C*03:02, C*03:03, and C*03:04 reacted very weakly with the 163LW-specific OK6H10 and OK6H12 (Table 4).

Table 5
Reactivities of MUL9E11 (IgG) and VTM9A10 (IgG) with 69AA eplets

		MUL9E11	69AA	Eplet															
			69	71	41	43	46	59	62	65	66	73	76	79	80	82	83	149	158	
Immunizing allele		B*55:01	A	A	A	P	E	Y	R	Q	I	T	E	R	N	R	G	A	A	Number of differences
	Antibody producer																			
		A*02:01	A	S	–	Q	–	–	G	R	K	–	V	G	T	–	–	–	–	7
		A*25:01	A	S	–	Q	–	–	–	R	N	–	–	–	I	L	R	T	–	7
		B*18:01	T	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
		B*51:01	T	T	–	–	–	–	–	–	–	–	–	–	I	L	R	–	–	3
		C*12:02	R	A	–	–	–	–	–	–	K	A	V	–	–	–	–	–	–	3
OL	GP	Panel																		
9658	15102	Positive control																		
18	100	Negative control																		
11646	17103	B*07:02	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
15705	12130	B*27:08	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
15343	17868	B*42:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
17460	18050	B*54:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
16680	18201	B*55:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
17459	17386	B*56:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
15076	10489	B*81:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
19188	nt	B*82:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
nt	18267	B*82:02	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
11234	nt	B*67:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	T	
nt	457	B*27:03	A	A	–	–	–	H	–	–	–	–	–	–	T	L	–	–	–	
3250	2348	B*27:05	A	A	–	–	–	–	–	–	–	–	–	–	T	L	–	–	–	
40	110	B*73:01	A	A	–	–	–	–	–	–	–	–	V	–	–	–	–	–	–	
13223	12306	B*15:16	A	A	–	–	A	–	–	R	N	–	–	–	I	L	R	–	–	
10528	10466	B*57:01	A	A	–	–	A	–	G	R	N	–	–	–	I	L	R	–	–	
13457	nt	B*57:03	A	A	–	–	A	–	G	R	N	–	–	–	I	L	R	–	–	
6694	14141	B*58:01	A	A	–	–	–	–	G	R	N	–	–	–	I	L	R	–	–	
19 ± 9	68 ± 34	Self Alleles																		
20 ± 9	90 ± 33	Other 69AA-negative alleles																		
		VTM9A10	69AA eplet																	
	Sequence positions		69	71	41	43	46	59	62	65	66	73	76	79	80	82	83	149	158	
Immunizing allele		B*07:02	A	A	A	P	E	Y	R	Q	I	T	E	R	N	R	G	A	A	Number of differences
	Antibody producer																			
		A*25:01	A	S	–	Q	–	–	–	R	N	–	–	–	I	L	R	T	–	7
		A*29:02	A	S	–	Q	–	–	L	R	N	–	A	G	T	–	–	–	–	7
		B*15:01	T	T	–	–	A	–	–	–	–	–	–	–	–	–	–	–	–	0
		B*44:02	T	T	T	–	–	–	–	–	–	–	–	–	T	L	R	–	–	4
		C*05:01	R	A	–	–	–	–	–	–	K	–	V	–	K	–	–	–	–	3
OL	GP	Panel																		
11034	15687	Positive control																		
5	87	Negative control																		
10674	17798	B*07:02	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
14587	17655	B*27:08	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
15186	18440	B*42:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
8944	17210	B*54:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
15124	17984	B*55:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
7397	13257	B*56:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
12193	15465	B*81:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
11123	nt	B*82:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
nt	11026	B*82:02	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
10752	nt	B*67:01	A	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	T	
9252	1763	B*73:01	A	A	–	–	–	–	–	–	–	–	V	–	–	–	–	–	–	
nt	9102	B*27:03	A	A	–	–	–	H	–	–	–	–	–	–	T	L	R	–	–	
11837	17072	B*27:05	A	A	–	–	–	–	–	–	–	–	–	–	T	L	R	–	–	
306	1005	B*15:16	A	A	–	–	A	–	–	R	N	–	–	–	I	L	R	–	–	
809	263	B*57:01	A	A	–	–	A	–	G	R	N	–	–	–	I	L	R	–	–	
490	nt	B*57:03	A	A	–	–	A	–	G	R	N	–	–	–	I	L	R	–	–	
366	781	B*58:01	A	A	–	–	–	–	G	R	N	–	–	–	I	L	R	–	–	
29 ± 26	77 ± 20	Self alleles																		
25 ± 9	82 ± 28	Other 69AA-negative alleles																		

These alleles have a residue difference in hidden position 52 (Table 7), but there are also differences in exposed positions 66 and 173 (Table 4). MUL9E11 is specific for the 69AA+self76E pair (Table 5). The nonreactive B*73:01 has 69AA+76V and it is possible that the hidden residue difference 77S → G plays a role. Altogether, however, these findings suggest that hidden residue differences appear to have no major effect on epitope reactivity with the mAbs in this study.

3.7. Molecular modeling of the topography of structural epitopes

Figure 1 shows the locations of specific eplets and surface residues within 15 Å on the molecular surface of informative structural models of crystallized HLA alleles. These illustrations offer estimates of the topography of structural epitopes consisting of residues contacted by the mAbs in this study. As described above, our analysis has provided some information about the locations of

Table 6
Reactivity of MUS4H4 (IgG) and KAL3D5 (IgG) with Bw4-related epitopes

				Sequence position	76	79	80	82	83	43	65	66	69	71	73	90	138	144	145	149	150	
				Immunizing allele		Eplet 80ERILR																
				B*49:01	E	R	I	L	R	P	Q	I	T	T	T	A	T	Q	R	A	A	Number of differences
				Antibody producer																		
				A*01:01	A	G	T	R	G	Q	R	N	A	S	—	D	M	K	—	—	V	9
				A*26:01	A	G	T	R	G	Q	R	N	A	S	—	D	M	—	—	T	—	8
				B*08:01	E	R	N	R	G	—	—	—	—	—	—	—	—	—	—	—	—	0
				B*27:05	E	R	T	L	R	—	—	—	A	A	—	—	—	—	—	—	—	2
				C*01:02	V	R	N	R	G	—	—	K	R	A	—	—	—	—	—	—	—	3
				C*07:02	V	R	N	R	G	—	—	K	R	A	A	D	—	—	—	—	—	5
MUS4H4 OL MFI	MUS4H4 GP MFI	KAL 3D5 OL MFI	KAL 3D5 GP MFI	Panel																		
14108	10434	10446	10086	Positive control																		
56	369	4	316	Negative control																		
23951	13574	13463	11612	B*49:01	E	R	I	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
23694	8964	13071	4570	B*15:13	E	R	I	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
23888	13537	15974	7947	B*38:01	E	R	I	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
23535	13662	18003	12009	B*51:01	E	R	I	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
24112	nt	20161	nt	B*51:02	E	R	I	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
23723	10645	16044	8572	B*52:01	E	R	I	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
24202	15858	20423	11916	B*53:01	E	R	I	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
22231	nt	16391	nt	B*59:01	E	R	I	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
24007	17645	18064	14917	B*15:16	E	R	I	L	R	—	R	N	A	A	—	—	—	—	—	—	—	
24109	15853	7354	9042	B*57:01	E	R	I	L	R	—	R	N	A	A	—	—	—	—	—	—	—	
24064	nt	13060	nt	B*57:03	E	R	I	L	R	—	R	N	A	A	—	—	—	—	—	—	—	
23576	18862	7538	13222	B*58:01	E	R	I	L	R	—	R	N	A	A	—	—	—	—	—	—	—	
24030	nt	9066	nt	A*23:01	E	R	I	L	R	Q	G	K	A	S	—	—	M	—	—	—	—	
nt	9730	nt	1431	A*23:02	E	R	I	L	R	Q	G	K	A	S	—	—	M	—	—	—	—	
23808	17620	9779	8607	A*24:02	E	R	I	L	R	Q	G	K	A	S	—	—	M	K	—	—	—	
24023	18614	10706	9080	A*24:03	E	R	I	L	R	Q	G	K	A	S	—	—	M	K	—	—	—	
24414	11838	13356	3883	A*25:01	E	R	I	L	R	Q	R	N	A	S	—	D	M	—	—	T	—	
24388	13852	13720	6727	A*32:01	E	R	I	L	R	Q	R	N	A	S	—	—	M	—	—	—	—	
23778	nt	24	nt	B*13:01	E	R	T	L	R	—	—	—	—	—	—	—	—	—	L	—	—	
23502	14792	151	1173	B*13:02	E	R	T	L	R	—	—	—	—	—	—	—	—	—	L	—	—	
24100	18629	92	1961	B*37:01	E	R	T	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
22433	6969	23	329	B*44:02	E	R	T	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
23884	12520	34	419	B*44:03	E	R	T	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
20840	15903	9	331	B*47:01	E	R	T	L	R	—	—	—	—	—	—	—	—	—	—	—	—	
nt	13390	nt	327	B*27:03	E	R	T	L	R	—	—	—	A	A	—	—	—	—	—	—	—	
24075	18546	9	383	B*27:05	E	R	T	L	R	—	—	—	A	A	—	—	—	—	—	—	—	
30 ± 3	470 ± 30	10 ± 7	331 ± 31	Average self alleles																		
41 ± 19	516 ± 154	11 ± 8	316 ± 50	Other alleles																		

surface residues associated with antibody binding. Here, we address 2 general issues relevant to our understanding of structural epitopes in context with eplets as functional epitopes.

First, crystallographic studies of different protein antigen–antibody complexes have shown a range of 15 to 25 epitope residues contacted by the CDRs of the antibody [5–7]. For the 5 eplets in this study, we have calculated from the models in Fig. 1 that eplets and their corresponding 15-Å^2 surface areas have an average of 33 residue locations (range 25–40). These numbers are higher than the general 15 to 25 range of contact residues in structural epitopes of experimentally tested protein antigens. As described above, residue substitutions in certain surface locations do not affect the binding of an epitope-carrying allele to a specific antibody, and it is possible that structural epitopes do not include these locations. Accordingly, the numbers of remaining residue locations range from 24 to 34, which is still higher than the expected number of contact residues. It should be pointed out that most residues within 15 Å of a given eplet are monomorphic and that the compositions of current Luminex panels do not permit further analysis. More detailed descriptions of structural epitopes seem possible by testing mAbs with alleles with informative point mutations [37,38] or, better yet, by analyzing crystallized HLA antigen–antibody complexes similar to that described by Ziegler's group [39]. Nevertheless, our model of surface residues within a 15-Å radius of a centrally located eplet offers a reasonable estimate of a structural epitope contacted by a specific HLA antibody.

Second, all structural models except 41T have HLA-bound peptide residues within the 15-Å radius of a given eplet (Fig. 1). This raises the question of whether they are relevant parts of structural epitopes in that they make significant contact with the antibody. Several studies have demonstrated that alloantibodies can recognize class I molecules in context with bound peptide [40–43]. Mulder and co-workers studied several human mAbs specific for epitopes presented by immunizing HLA-A2 antigens. By loading recombinant monomeric HLA-A2 molecules with 12 different peptides, they demonstrated that certain peptides markedly reduced the reactivity of some mAbs, whereas other mAbs were unaffected by any of these peptides [43]. Peptide-dependent alloantibodies appear to have 1 CDR required for HLA epitope recognition, whereas peptide residues react with a second CDR critical for stabilizing antigen binding [43]. Our study could not determine whether any of the mAbs described above is peptide dependent. One might expect that the alleles in the Luminex panels have different repertoires of multiple peptides. A peptide-dependent alloantibody may interact with certain residues shared between a group of peptides bound to a given allele, whereas the remaining peptides are nonreactive. Such differential reactivity would lead to lower MFI values because only a proportion of allelic molecules on a given Luminex bead have the proper peptide residues necessary for binding with peptide-dependent alloantibody.

Table 7
Effects of residue differences in antibody-inaccessible positions on the reactivity of alleles with epitope-specific mAbs

mAb	Epitope	Residue differences without significant effects on allele reactivity with mAb								Differences on alleles with lower reactivity				
ROU9A6	41T	32L→Q	45K→M											
KAM3H9	80NRG	11S→V	12V→M	21R→H	70Q→N or K	74D→Y	77G→S	94T→I						
OUW4F11		95L→W	97S→R or W, T	144D→N	116Y→F or S, L, D	143T→S	147W→L	152E→V						
OK6H10	163LW	9Y→H	32Q→L	63E→N	67S→C or M, Y	70N→S or Q				52I→V				
OK6H12		103V→L	113H→Y	152E→V	156L→W or R	171Y→H								
MUL9E11	69AA + s76E	11A→S	12V→M	24A→S or T	32Q→L	45E or G, M, T	63N→E	67Y→C or M	77S→G					
		70Q→K or S	74D→Y	77S→D or N	81L→A	94T→I	95W→L or I	97T→S or R, N, V						
		113H→Y	114N→D or H	116L→Y or F, D, S	147W→L	152E→V	156L→R or D							
VTM9A10	69AA	9Y→H	11S→A	12V→M	24S→A or T	32Q→L	45E→G	63E→N		45E→M or T	67Y→M	70Q→S	74D→Y	77S→N
		67Y→C	70Q→K	77S→D	95L→W	97S→T or R	113H→Y	114D→N		81L→A	94T→I	97S→V	116Y→S	
		116Y→L or F, D	143T→S	147W→L	152E→V	156R→D or L								
KAL3D5	80ERILR	9H→S or F, Y	11A→S	12M→V	24T→A	67S→V or M, C, F	70N→H or S	74Y→D						
		77N→S	94T→I	95L→W	97R→M or T	114N→Q or D	116L→Y or D, S	152E→V						

4. Discussion

This analysis has increased our understanding of the immunogenicity (*i.e.*, the ability to induce an antibody response) and antigenicity (*i.e.*, the reactivity with specific antibody) of HLA class I epitopes. Human monoclonal antibodies are ideal for these studies because, by definition, each of them is specific for a single epitope. Their reactivity patterns with HLA allele panels indicate that the immunizing antigen and reactive alleles share an epitope that can be predicted by HLAMatchmaker from the HLA types of immunizer and antibody producer. In these studies, we distinguished between structural epitopes, which, by definition, cover that part of the molecular surface contacted by all CDR loops of antibody and centrally located functional epitopes, which consist of patches of residues that dominate the strength and specificity of antibody binding. Because HLA antibodies are generally specific for eplets, it appears likely that eplets are essential elements of functional epitopes located within structural epitopes that we estimate have surface areas in the 15 Å radius range. This perspective offers a new approach to study the structural basis of HLA epitope immunogenicity and antigenicity.

The nonself–self paradigm of HLA epitope immunogenicity is based on the hypothesis that HLA antibodies originate from B cells with self-HLA Ig receptors that recognize mismatched eplets within structural epitopes on immunizing antigens [19,44]. It predicts that, except for the mismatched eplet, the remainder of a structural epitope on the immunizing antigen must be virtually identical to a corresponding self HLA epitope of the antibody producer. Our findings are consistent with this notion. For each epitope described above, we could identify a self configuration in the antibody producer that has no surface residue difference with the immunizing allele. This suggests that the humoral alloresponse requires that the immunizing HLA epitope have a similar structure as the self epitope of the antibody producer. Conversely, a lack of structural similarity may prevent an antibody response, which may explain why sensitized patients have restricted patterns of antibody specificity after being exposed to multiple epitope mismatches. The nonself–self paradigm of HLA immunogenicity might have useful potential in predicting antibody responses to HLA mismatches, but of course this must be verified in the clinical setting.

This study addressed antibodies reacting with epitopes with frequencies ranging from 45 to 89%. Because many alleles share such epitopes, it was possible to study the influence of amino acid differences on epitope antigenicity (*i.e.*, antibody binding). Luminex assay results are presented as MFI values, which reflect the proportions of allele-carrying beads that bind antibody. High MFI values comparable to positive controls indicate high proportions and extremely low MFI values comparable to those with self alleles of the antibody producer and negative controls indicate a lack of binding with antibody. Often, many MFI values are in the intermediate or low range, suggesting that a smaller distribution of beads with that allele bind with antibody. Because antibody binding involves all CDRs interacting with the structural epitope, we have raised the question of whether lower MFI values of certain epitope-carrying alleles are related to amino acid differences with the immunizing allele. The tables present several examples of 3 scenarios.

First, a residue difference has no significant effect on MFI; examples are the 46A versus 46E difference of B13 alleles reacting with the 41T-specific ROU9A6 (Table 1) and the 69T71T versus 69A71A difference for a larger group of alleles reacting with the 80NRG-specific KAM3H9 (Table 2). It seems apparent that these residue locations do not play significant roles as binding sites, because they may lie outside the actual structural epitope, or that CDR loops have considerable flexibility that allows a single antibody molecule to interact with a large number of related proteins [45].

Second, extremely low MFI values are associated with distinct residue differences that apparently abolish antibody binding. As an example, the 69AA-carrying B*73:01 has a 76V versus 76E difference and did not react with the 69AA-specific MUL9E11 (Table 4). Apparently, certain locations such as position 76 on the structural epitope require identical residues shared between immunizer and antibody producer; they serve as critical contact sites that interact with the antibody [16].

Third, certain alleles with intermediate and low MFI values have the same residue differences; examples are B*46:01 and a group of HLA-C alleles with 69R rather than 69A, with intermediate MFI values with the 80NRG-specific KAM3H9 (Table 2), and the very low reactivity of the 69AA-specific VTM9A10 with the 69AA-carrying B*15:16, B*57:01, B*57:03, and B*58:01, which have 65R and 66N rather than 65Q and 66I (Table 5). Apparently, these residue differences seem to affect antibody binding but do not completely abolish it. Clinically speaking, how would one interpret the mismatch acceptability of specific epitope-carrying alleles with low MFI values that are significantly higher than those with the self alleles of the antibody producer?

This study also addressed the possibility that hidden residues below the molecular surface may affect the binding of epitope-carrying alleles to specific antibodies. Although such residues are not antibody accessible, they might influence the conformations of epitopes and critical sites that contact the antibody. However, for the 5 eplets studied here and their respective 15 Å radius regions, we did not obtain any convincing evidence of a significant effect.

In conclusion, this analysis of antibody reactivity has increased our understanding of the structural basis of HLA epitopes and the interpretation of MFI values with alleles in Luminex panels. Although this study has a potential limitation in that the Luminex panels did not have all informative alleles, including point mutants, the data demonstrate that certain surface residues within a 15-Å radius appear to affect antibody binding, whereas others have no effect. This type of information may refine the assessment of HLA epitope mismatch acceptability in the clinical setting.

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