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New HLA class I epitopes defined by murine monoclonal antibodies

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ABSTRACT

This study defines 10 epitopes by murine monoclonal antibodies, of which seven are new and three were previously defined by alloantibodies. Of particular interest, three antibodies reacted with almost all Bw4-associated antigens except that each was negative with one or two of the antigens. One was negative with B13, one negative with A25, and another negative with B13 & A24 antigens. These monoclonal antibodies exhibited reactivity contrary to Bw4 allosera, which typically react with all Bw4-associated antigens. All monoclonal antibodies were tested with a panel of 97 human leukocyte antigen (HLA) class I (A, B, and Clocus) rHLA single antigens (SA) individually coupled to different microsphere beads. Identifying HLA antigens sharing distinct epitopes can be helpful when selecting patient–donor transplantation pairs, explaining antibodies against rare specificities, or against non–donor-specific antigens (NDSA). This study adds seven new HLA class I epitopes to 103 already defined epitopes and provides more evidence that a monoclonal antibody and alloantibody can target the same epitope. The fact that mAbs can target the same epitopes targeted by allosera, makes mAbs useful in studies of cross-reactivity in HLA.

1. Introduction

Anti–human leukocyte antigen (HLA) antibodies infrequently react against one single HLA antigen because the epitopes they target are normally shared by more than one antigen. The use of the single antigen (SA) beads has been essential in the definition of 103 epitopes on HLA class I antigens targeted by monoclonal antibodies (mAbs) and by purified alloantibodies that have been absorbed from sera by recombinant HLA cell lines (rHLA) then eluted [1]. In addition, we have recently defined 96 epitopes targeted by antibodies found in the sera of healthy normal male subjects [2]. In this study, we define 10 epitopes all targeted by monoclonal antibodies (mAbs). Of these epitopes, seven are new and three were previously defined by alloantibodies. Three mAbs target epitopes that are shared by the Bw4-associated antigens except that one was negative with B13, one negative with A25, and another negative with B13 and A24 antigens. The reactivity of these mAbs exhibited reactions contrary to what is observed with allosera. Typically allosera react with all Bw4-associated antigens, suggesting that allosera contain many antibodies, all together, reacting with all Bw4-associated antigens. Identifying HLA antigens sharing distinct epitopes can be useful when selecting patient–donor transplantation pairs, explaining antibodies against rare specificities, or against non–donor-specific antigens. Rare and nondonor specificities are detected by antibodies targeting the same epitope shared with mismatched donor antigens. The fact that mAbs can target the same epitopes targeted by allosera makes mAbs useful in studies of cross-reactivity in HLA.

2. Subjects and methods

2.1. Monoclonal antibodies

Murine mAbs produced by conventional methods of hybridoma cell lines were used in this study and their HLA specificities were first determined by complement dependent cytotoxicity (CDC) assay. Monoclonal antibodies were tested as diluted ascites (1:10 to 1:50,000). Because the mAbs were not purified, protein concentrations of the final dilutions were not determined. The mAbs used for this study were from repeatedly cloned hybridoma cell lines.

2.2. Testing assay and data analysis

Monoclonal antibodies were tested with a panel of 97 HLA class I (A, B, and C-locus) rHLA SA individually coupled to different microsphere beads, and with negative and positive control beads (LABScreen beads: LS1A04; One Lambda, Canoga Park CA)) according to the method described by Pei et al. [3].

LABScreen assays, data analysis, and amino acid mapping for epitope definitions were as previously described in detail [4].

3. Results

Table 1 lists 10 HLA class I epitopes identified by mAbs. Epitopes are defined by one, two, or three amino acids (aa) and are numbered according to the method we adopted in our earlier study [4]. Epitopes 43, 247, 248, 249, 250, 422, and 423 are newly defined. Epitopes 19, 31, and 225 are examples of epitopes defined by mAbs

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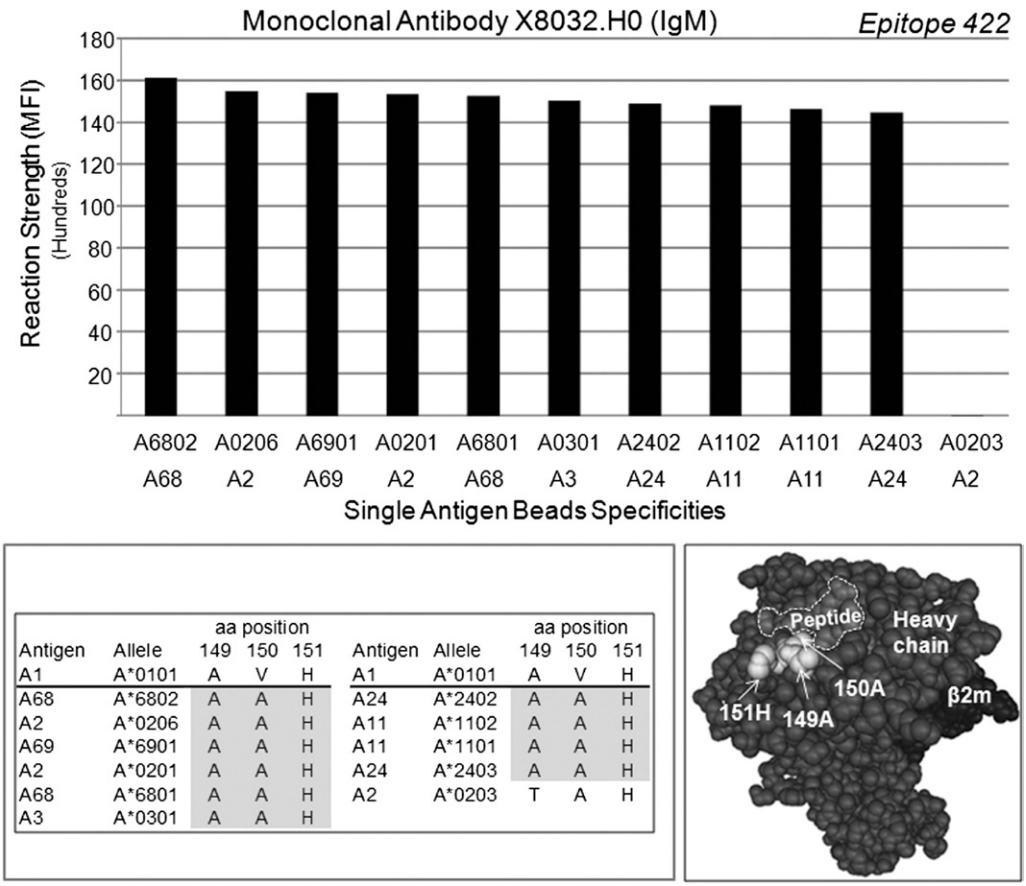
Table 1
HLA class I epitopes identified by murine monoclonal antibodies

mAb	HLA of immunizing cell	Single antigen beads with positive reactions	Epitope no.	New/ alloserum	Position and unique aa for possible epitope sites
Z3633.T0 IgM	A2, 11, B62, 35, Cw9, 4	B1502(B75), B1301, B1512(B76), B1302, B1513(B77), B5703, B5701, B1501(B62), B1511(B75), B1516(B63), B4601	43	New	46A
0131HA3-7 IgG	A2, 69, B27, 44, Cw2	A0201, A6901, A0203, A0206, A2902, 3601, A2901, A3101, A3303, A3402, A2403, A3001, A3401, A6601, A6602, A6802, A2501, A1102, A4301, A2601, A1101, A6801, A3002, A3301, A0301	247	New	109F+166E/109F+167W
X8342. H0 IgM	A1, B13,64, Cw6, 8	A0101, A3601, A1102, A1101, A0301	248	New	62Q+151H
0473HA IgG	A2, 26, B57, Cw6	A*2301, A*2402, A*2403, A25, A*3201, B77, B*1516, B*2705, B*3701, B*3801, B*4402, B*4403, B*4701, B*4901, B*5101, B*5102, B*5201, B*5202, B*5301, B*5701, B*5703, B*5801, B*5901 (B*1301 and B*1302 negative)	249	New	82L+145R/83R+145R
548HA1-4 IgM	A1, 26, B27, 37, Cw2, 6	A*2301, A*2402, A*2403, A*3201, B*1301, B*1302, B77, B*1516, B*2705, B*3701, B*3801, B*4402, B*4403, B*4701, B*4901, B*5101, B*5102, B*5201, B*5202, B*5301, B*5701, B*5703, B*5801, B*5901 (A2501 negative)	250	New	82L+90A/83R+90A
X8032. H0 IgM	A2,3, B7, 62	A6802, A206, A6901, A0201, A6801, A0301, A2402, A1102, A1101, A2403	422	New	149A+150A+151H
Z1153 IgM	A3, B65, Cw8[a]	A*2301, A25, A*3201, B77, B*1516, B*2705, B*3701, B*3801, B*4402, B*4403, B*4701, B*4901, B*5101, B*5102, B*5201, B*5202, B*5301, B*5701, B*5703, B*5801, B*5901 (A2402, A2403, B1301, B1302 negative)	423	New	83R +144Q+145R
0189HA IgM	A2, 24, B54, 67, Cw1, 7	A0206, A2402, A6802, A6801, A2403, A6901, A2301, A0201, A0203	19	Alloserum	127K
0273HA IgM	A31, B62, Cw1, 10	A3001, A3002, A3101	31	Alloserum	56R
0059HA	A26, B8, Cw7	B0801, B5901	225	Alloserum	(67F)+163T

Columns, left to right, show the following: monoclonal antibody (mAb) ID; HLA phenotype of the immunizing cell; HLA alleles that share the epitope; epitope number; whether the epitope is new or it has been previously defined by allosera; and amino acids and their positions that define the epitope.
[a]Homozygous for all three loci.

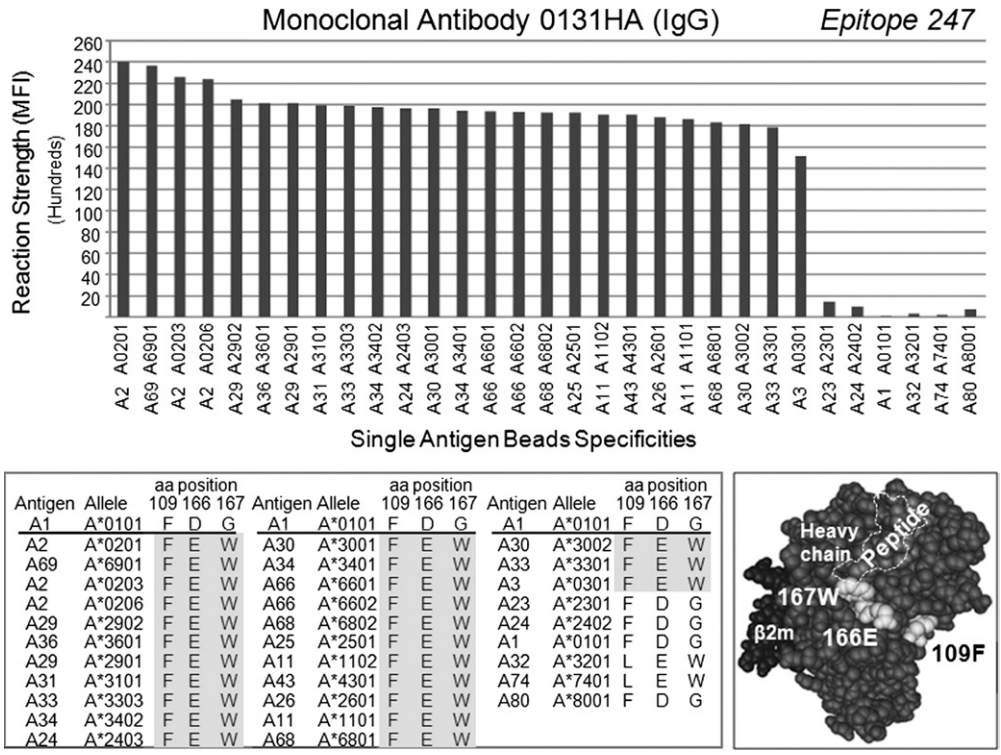
as well as with antibodies purified from allosera reported in our earlier study.

Actual fluorescence data for some of the HLA antigens sharing epitopes are shown in Figs. 1–6. Figure 1 shows the reactions of mAb X8032.H0 with A-locus HLA antigens that share amino acids alanine (A), alanine (A), and histidine (H) at positions 149, 150, and 151, respectively. This combination defines epitope 422 (Table 1). Although this mAb reacts positively with A0201 and A0206, it did



Antigen	Allele	aa position 149	150	151	Antigen	Allele	aa position 149	150	151
A1	A*0101	A	V	H	A1	A*0101	A	V	H
A68	A*6802	A	A	H	A24	A*2402	A	A	H
A2	A*0206	A	A	H	A11	A*1102	A	A	H
A69	A*6901	A	A	H	A11	A*1101	A	A	H
A2	A*0201	A	A	H	A24	A*2403	A	A	H
A68	A*6801	A	A	H	A2	A*0203	T	A	H
A3	A*0301	A	A	H					

Fig. 1. Monoclonal antibody X8032. H0 reacts with A-locus HLA antigens sharing the amino acids alanine (A), alanine (A), and histidine (H) at positions 149, 150, and 151, respectively. All three aa combined define epitope 422 (Table 1). Although this mAb reacts positively with A0201 and A0206, it reacts negatively with A0203, which has the aa threonine (T) instead of alanine at 149.

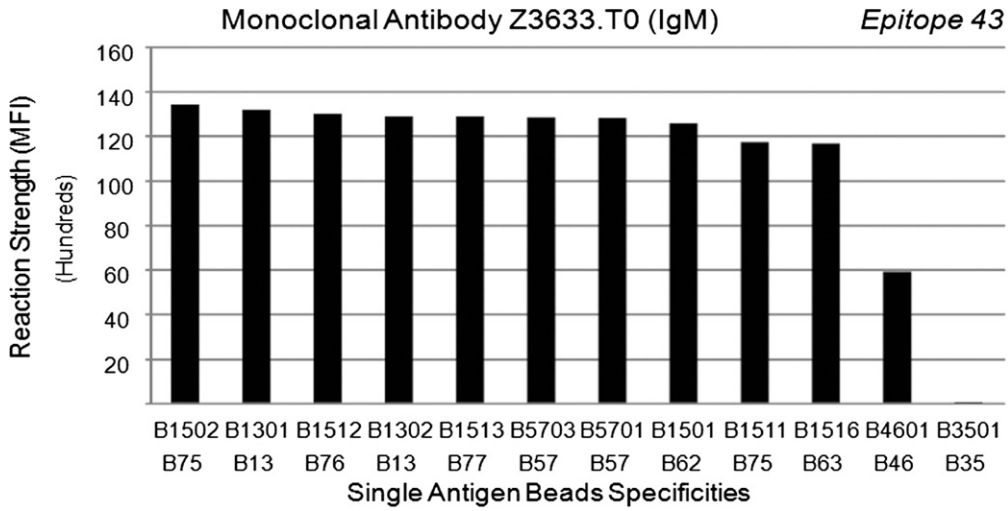


Antigen	Allele	aa position 109	166	167
A1	A*0101	F	D	G
A2	A*0201	F	E	W
A69	A*6901	F	E	W
A2	A*0203	F	E	W
A2	A*0206	F	E	W
A29	A*2902	F	E	W
A36	A*3601	F	E	W
A29	A*2901	F	E	W
A31	A*3101	F	E	W
A33	A*3303	F	E	W
A34	A*3402	F	E	W
A24	A*2403	F	E	W

Antigen	Allele	aa position 109	166	167
A1	A*0101	F	D	G
A30	A*3001	F	E	W
A34	A*3401	F	E	W
A66	A*6601	F	E	W
A66	A*6602	F	E	W
A68	A*6802	F	E	W
A25	A*2501	F	E	W
A11	A*1102	F	E	W
A43	A*4301	F	E	W
A26	A*2601	F	E	W
A11	A*1101	F	E	W
A68	A*6801	F	E	W

Antigen	Allele	aa position 109	166	167
A1	A*0101	F	D	G
A30	A*3002	F	E	W
A33	A*3301	F	E	W
A3	A*0301	F	E	W
A23	A*2301	F	D	G
A24	A*2402	F	D	G
A1	A*0101	F	D	G
A32	A*3201	L	E	W
A74	A*7401	L	E	W
A80	A*8001	F	D	G

Fig. 2. Monoclonal antibody 0131HA reacts with A-locus HLA antigens sharing the aa phenylalanine (F), Glutamic acid (E), and tryptophan (W) at positions 109, 166, and 167, respectively. Either of the two aa combinations 109F and 166E or 109F and 167W defines epitope 247 (Table 1). Negative A-locus antigens shown do not share either of the two combination aa and thus do not have the same epitope.



Antigen	Allele	aa position 46
B7	B*0702	E
B75	B*1502	A
B13	B*1301	A
B76	B*1512	A
B13	B*1302	A
B77	B*1513	A
B57	B*5703	A
B57	B*5701	A

Antigen	Allele	aa position 46
B7	B*0702	E
B62	B*1501	A
B75	B*1511	A
B63	B*1516	A
B46	B*4601	A
B52[a]	B*5202	A
B35	B*3501	E

[a] allele not present on SA beads panel

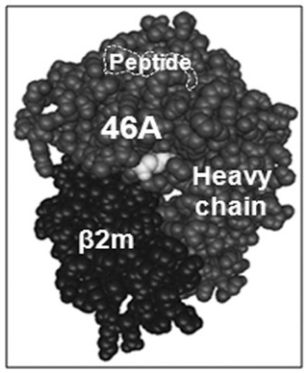
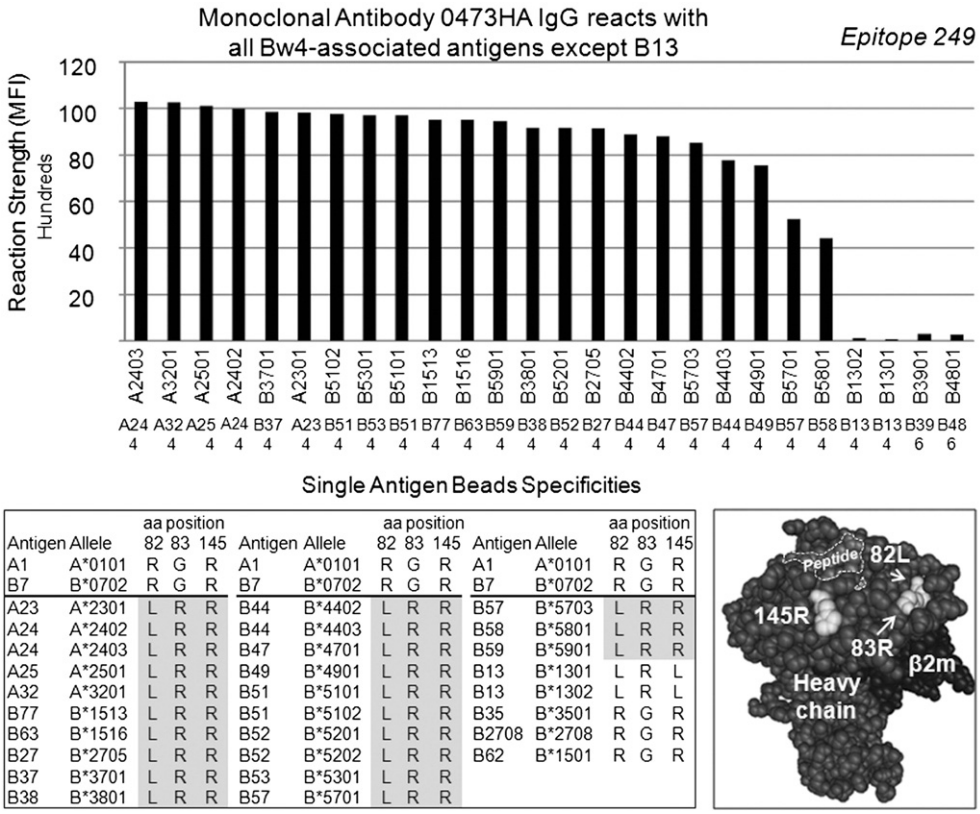
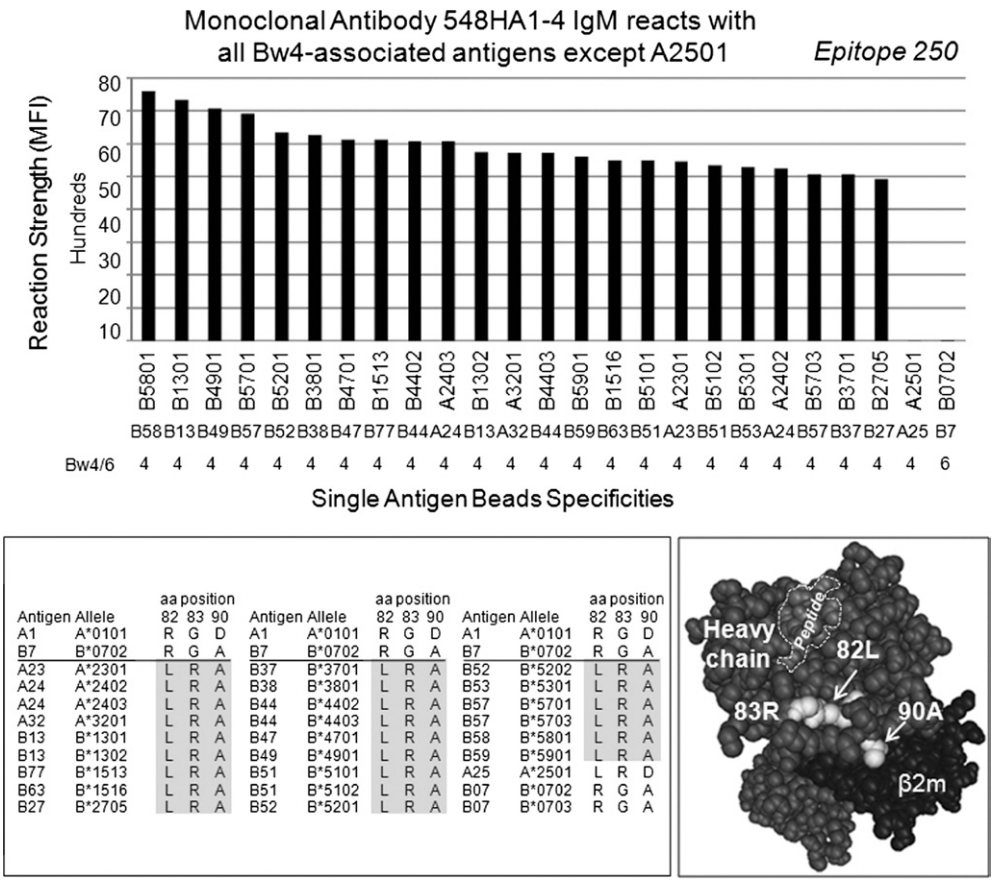


Fig. 3. Monoclonal antibody Z3633.T0 is positive against 11 B-locus antigens sharing the aa alanine (A) at position 46. None of the other antigens, negative with this mAb, in the SA panel has the aa alanine at this position thus 46A defines the target epitope 43 (Table 1).



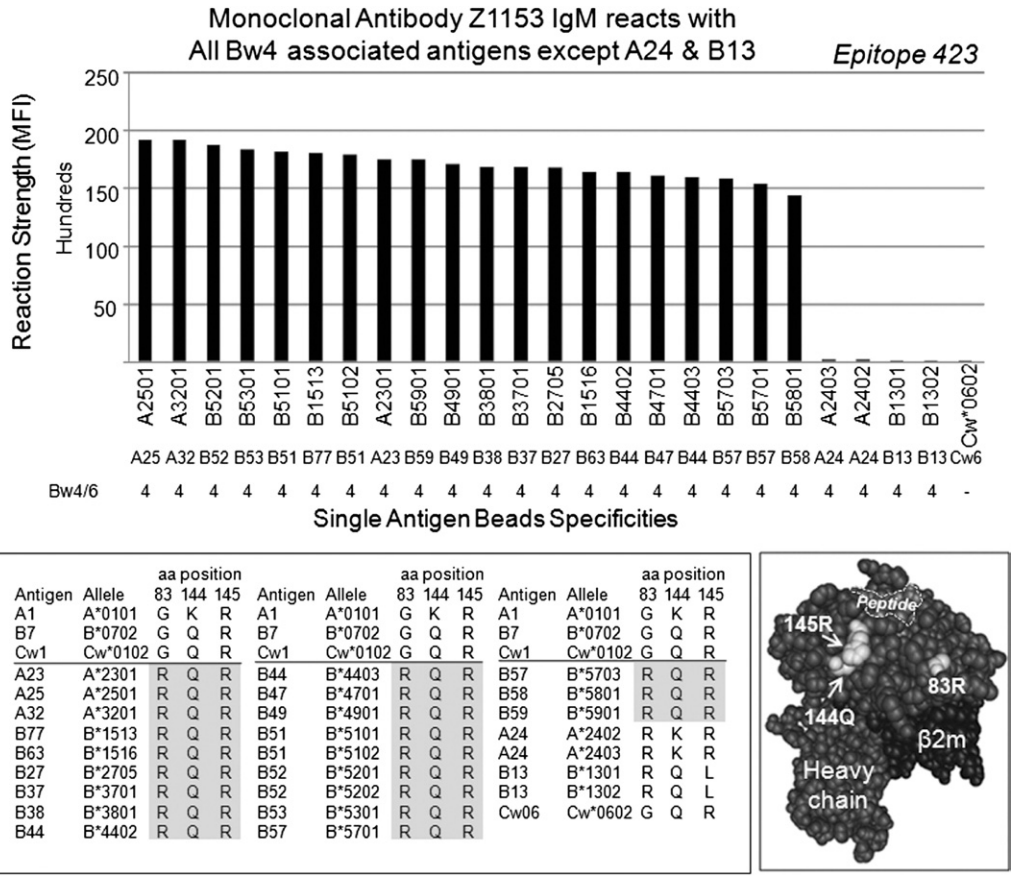
Antigen	Allele	aa position 82	83	145	Antigen	Allele	aa position 82	83	145	Antigen	Allele	aa position 82	83	145
A1	A*0101	R	G	R	A1	A*0101	R	G	R	A1	A*0101	R	G	R
B7	B*0702	R	G	R	B7	B*0702	R	G	R	B7	B*0702	R	G	R
A23	A*2301	L	R	R	B44	B*4402	L	R	R	B57	B*5703	L	R	R
A24	A*2402	L	R	R	B44	B*4403	L	R	R	B58	B*5801	L	R	R
A24	A*2403	L	R	R	B47	B*4701	L	R	R	B59	B*5901	L	R	R
A25	A*2501	L	R	R	B49	B*4901	L	R	R	B13	B*1301	L	R	L
A32	A*3201	L	R	R	B51	B*5101	L	R	R	B13	B*1302	L	R	L
B77	B*1513	L	R	R	B51	B*5102	L	R	R	B35	B*3501	R	G	R
B63	B*1516	L	R	R	B52	B*5201	L	R	R	B2708	B*2708	R	G	R
B27	B*2705	L	R	R	B52	B*5202	L	R	R	B62	B*1501	R	G	R
B37	B*3701	L	R	R	B53	B*5301	L	R	R					
B38	B*3801	L	R	R	B57	B*5701	L	R	R					

Fig. 4. Anti-Bw4 mAb 0473HA recognizes epitope 249 (Table 1), which is defined by the combination of aa leucine (L) and arginine (R) at positions 82 and 145, respectively, or by the two amino acids 83 and 145R and either combination is shared by all Bw4-associated antigens except B1301 and B1302.



Antigen	Allele	aa position 82	83	90	Antigen	Allele	aa position 82	83	90	Antigen	Allele	aa position 82	83	90
A1	A*0101	R	G	D	A1	A*0101	R	G	D	A1	A*0101	R	G	D
B7	B*0702	R	G	A	B7	B*0702	R	G	A	B7	B*0702	R	G	A
A23	A*2301	L	R	A	B37	B*3701	L	R	A	B52	B*5202	L	R	A
A24	A*2402	L	R	A	B38	B*3801	L	R	A	B53	B*5301	L	R	A
A24	A*2403	L	R	A	B44	B*4402	L	R	A	B57	B*5701	L	R	A
A32	A*3201	L	R	A	B44	B*4403	L	R	A	B57	B*5703	L	R	A
B13	B*1301	L	R	A	B47	B*4701	L	R	A	B58	B*5801	L	R	A
B13	B*1302	L	R	A	B49	B*4901	L	R	A	B59	B*5901	L	R	A
B77	B*1513	L	R	A	B51	B*5101	L	R	A	A25	A*2501	L	R	D
B63	B*1516	L	R	A	B51	B*5102	L	R	A	B07	B*0702	R	G	A
B27	B*2705	L	R	A	B52	B*5201	L	R	A	B07	B*0703	R	G	A

Fig. 5. Anti-Bw4 mAb 548HA1-4 recognizes epitope 250 (Table 1), which is defined by the aa combination leucine (L) and arginine (R) at positions 82 and 90, respectively, or by 83R and 90A, and either combination is shared by all Bw4-associated antigens except A2501.



Antigen	Allele	aa position 83	144	145
A1	A*0101	G	K	R
B7	B*0702	G	Q	R
Cw1	Cw*0102	G	Q	R
A23	A*2301	R	Q	R
A25	A*2501	R	Q	R
A32	A*3201	R	Q	R
B77	B*1513	R	Q	R
B63	B*1516	R	Q	R
B27	B*2705	R	Q	R
B37	B*3701	R	Q	R
B38	B*3801	R	Q	R
B44	B*4402	R	Q	R

Antigen	Allele	aa position 83	144	145
A1	A*0101	G	K	R
B7	B*0702	G	Q	R
Cw1	Cw*0102	G	Q	R
B44	B*4403	R	Q	R
B47	B*4701	R	Q	R
B49	B*4901	R	Q	R
B51	B*5101	R	Q	R
B51	B*5102	R	Q	R
B52	B*5201	R	Q	R
B52	B*5202	R	Q	R
B53	B*5301	R	Q	R
B57	B*5701	R	Q	R

Antigen	Allele	aa position 83	144	145
A1	A*0101	G	K	R
B7	B*0702	G	Q	R
Cw1	Cw*0102	G	Q	R
B57	B*5703	R	Q	R
B58	B*5801	R	Q	R
B59	B*5901	R	Q	R
A24	A*2402	R	K	R
A24	A*2403	R	K	R
B13	B*1301	R	Q	L
B13	B*1302	R	Q	L
Cw06	Cw*0602	G	Q	R

Fig. 6. Anti-Bw4 mAb Z1153 recognizes epitope 423 (Table 1), which is defined by the aa arginine (R), glutamine (Q), and arginine (R) at positions 83, 144, and 145, respectively, and is shared by all Bw4-associated antigens except A2402, A2403, B1301, and B1302.

not react with A0203, which has the aa threonine (T) instead of alanine at 149.

Figure 2 illustrates the reactions of mAb 0131HA with A-locus HLA antigens that share the amino acids phenylalanine (F), glutamic acid (E), and tryptophan (W) at positions 109, 166, and 167, respectively. Either combination 109F + 166E or 109F + 167W define epitope 247 (Table 1). It is clear that the negative A-locus antigens shown in the figure do not share either of these aa combinations, and thus do not have the same epitope.

Figure 3 shows 11-B locus HLA antigens reacting with mAb Z3633.T0. All 11 positive antigens share the aa alanine (A) at position 46. The remaining 85 single antigens in the panel are negative, and none have the aa alanine at this position; thus 46A defines the target epitope 43 (Table 1). Figures 4–6 illustrate the reaction patterns for three mAbs each reacting with most, but not all, of the Bw4-associated antigens. The first mAb 0473HA reacts with all Bw4-associated antigens except B1301 and B1302. This mAb recognizes epitope 249 (Fig. 4, Table 1) which is defined by the aa leucine (L) and arginine (R) at positions 82 and 145, respectively or by the two amino acids 83 and 145 R. The second mAb 548HA1-4 reacts with Bw4-associated antigens except A2501. This mAb recognizes epitope 250 (Fig. 5, Table 1) defined by leucine (L) and arginine (R) at positions 82 and 90, respectively or by 83R and 90A. The third mAb Z1153 is positive with Bw4-associated antigens except A2402, A2403, B1301 and B1302. The mAb recognizes epitope 423 (Fig. 6, Table 1) defined by arginine (R), glutamine (Q), and arginine (R) at positions 83, 144, and 145, respectively. Figure 7 shows comparison of the reaction patterns of one alloserum and the three monoclonal antibodies with Bw4-associated HLA antigens. It is clear that the alloserum reacts with all Bw4-associated antigens, whereas mAbs 0473HA, 548HA.1-4, and Z1153 are negative with B13, A25, and A24 and B13, respectively.

4. Discussion

Cross-reactivity in HLA has been observed since the dawn of the HLA field [5,6]. Cross-reacting groups (CREG) of antigens [7–10], known for almost 40 years, have proved useful in the interpretation of the non–donor-specific antibodies (NDSA) that are often observed in the sera of transplant patients. After the structure of HLA became known [11], it was possible to map epitopes, based on aa substitutions, which are the target of the HLA antibodies. Earlier studies offered epitope definitions derived from theoretic analysis based exclusively on shared amino acids among HLA alleles. However, our use of single antigen beads made it possible to offer experimental evidence to identify the exact antigens that share an epitope. We have already shown that a monoclonal or alloantibody shows positive reactions with only those antigens that exclusively share the epitope targeted by the antibody [1,4].

This study adds seven new HLA class I epitopes identified by monoclonal antibodies. Three epitopes, previously defined by alloantibodies, provide further evidence that monoclonal and alloantibodies can target the same HLA epitopes. Three mAbs against Bw4-associated antigens were particularly interesting in that none of them reacted with all HLA-A and B antigens that share the Bw4 epitope. Two Bw4 allosera were tested and neither one showed negative reactions with any of the Bw4-associated antigens. This may suggest that a Bw4 alloserum has a single antibody that targets the same epitope on all Bw4-associated antigens or that the

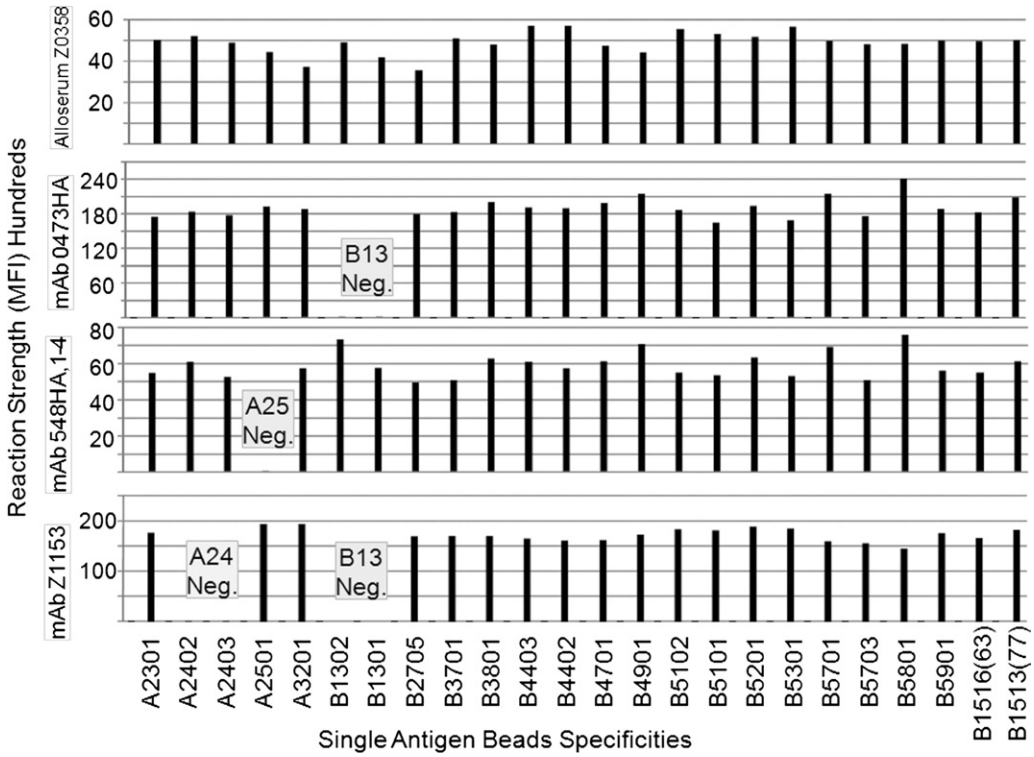


Fig. 7. Reaction patterns of one alloserum and three monoclonal antibodies with Bw4-associated HLA antigens. It is clear that the alloserum reacts with all Bw4-associated antigens whereas mAbs 0473HA, 548HA.1-4, and Z1153 react negatively with B13, A25, and A24 and B13, respectively.

serum has more than one antibody each targeting a different epitope on different groups of Bw4-associated antigens. It has been the experience in the past that Bw4 allosera that react exactly the same have been difficult to find. The new finding that there are at least three types of Bw4 monoclonal antibodies suggest that allosera against Bw4 may have been similarly different.

We have previously stated that NDSA in allosera are the same antibodies that react with donor-specific and non–donor-specific antigens sharing the same epitope. Similarly, antibodies against rare antigens are antibodies targeting epitopes shared among the rare antigen and antigens with higher frequencies [1,2,4]. Therefore, defining the HLA immunogenic epitopes in organ transplantation or blood transfusion is essential in the correct interpretation of allosera specificities. In a particular, organ transplant rejection case in which testing was done for the ABDR antigens only, the rare specificities (B46 and B73) were observed. Further typing and screened for the C-locus antigens revealed a Cw7 antigen mismatch and the serum specificities B46, B73, Cw1, Cw7, Cw8, Cw9, Cw10, Cw12, and Cw14. It is reasonable to suggest that Cw7 was the immunogen and the antibody that was produced is targeting epitope 246 [1] which is shared among B46, B73, Cw1, Cw7, Cw8, Cw9, Cw10, Cw12, Cw14, and Cw16 antigens (unpublished data).

References

[1] El-Awar NR, Akaza T, Terasaki PI, Nguyen A. Human leukocyte antigen class I epitopes: Update to 103 total epitopes, including the C locus. Transplantation 2007;84:532–40.
[2] El-Awar N, Terasaki PI, Nguyen A, Sasaki N, Morales-Buenrostro LE, Saji H, et al. Epitopes of human leukocyte antigen class I antibodies found in sera of normal healthy males and cord blood. Hum Immunol 2009;70:844–53.
[3] Pei R, Lee JH, Shih NJ, Chen M, Terasaki PI. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. Transplantation 2003;75:43–9.
[4] El-Awar N, Lee JH, Tarsitani C, Terasaki PI. HLA class I epitopes: Recognition of binding sites by mAbs or eluted alloantibody confirmed with single recombinant antigens. Hum Immunol 2007;68:170–80.
[5] Dausset J. Iso-Leuco-anticorps. Acta Haematol 1958;20:156–66.
[6] Van Rood JJ, Van Leeuwen A. Leukocyte grouping. A method and its application. J Clin Invest 1963;42:1382–90.
[7] Rodey GE, Sturm B, Aster RH. Cross-reactive HL-A antibodies. Separation of multiple HL-A antibody specificities by platelet adsorption and acid elution. Tissue Antigens 1973;3:63–9.
[8] Rodey GE, Fuller TC. Public epitopes and the antigenic structure of the HLA molecules. Crit Rev Immunol 1987;7:229–67.
[9] Fuller AA, Rodey GE, Parham P, Fuller TC. Epitope map of the HLA-B7 CREG using affinity-purified human alloantibody probes. Hum Immunol 1990;28:306–25.
[10] Fuller AA, Trevithick JE, Rodey GE, Parham P, Fuller TC. Topographic map of the HLA-A2 CREG epitopes using human alloantibody probes. Hum Immunol 1990;28:284–305.
[11] Bjorkman PJ, Strominger JL, Wiley DC. Crystallization and X-ray diffraction studies on the histocompatibility antigens HLA-A2 and HLA-A28 from human cell membranes. J Mol Biol 1985;186:205–10.