Human Leukocyte Antigen Class I Epitopes: Update to 103 Total Epitopes, Including the C Locus

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Background. Epitopes of human leukocyte antigen (HLA) are the sites to which the antibodies bind. We identify here 103 HLA class I epitopes shared by groups of class I antigens. In particular, our emphasis was on identifying epitopes exclusive to the C-locus antigens or interlocus epitopes among A, B, and C antigens. The use of monoclonal antibodies or alloantibodies eluted from HLA recombinant single antigen cell lines tested with a panel of single antigen beads have proved very useful in the identification of the epitopes.
Methods. Alloantibodies absorbed onto then eluted from HLA single antigen cell lines and monoclonal antibodies were tested with a panel of 95 A-, B-, and C- single antigen beads and the HLA specificities determined. Each epitope was defined by amino acids shared exclusively by the positive antigens for each antibody.
Results. In addition to the 58 A and B class I epitopes identified in an earlier study, we add 45 more new A, B, C epitopes including, for the first time, epitopes found on C locus antigens.
Conclusion. Beads bearing single antigens tested with monoclonal or eluted alloantibodies proved very powerful in identifying epitopes shared among HLA antigens. These epitopes are the targets of the antibodies. Antibody specificities to nondonor-specific antigens, often found in sera of transplant patients, can now be understood as reactions to epitopes shared with the donor specific antigens. The importance of identifying these epitopes is that they may be the "transplantation antigens" responsible for antibody-mediated transplant rejection.

Keywords: HLA epitopes, HLA antibodies, HLA crossreactions, Eluted antibodies, Recombinant HLA class I antigens.

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Recently we identified 58 epitopes shared among A and B human leukocyte antigen (HLA) class I antigens by monoclonal antibodies (mAbs) or allo antibodies that were absorbed and eluted from HLA recombinant single antigen cell lines (rHLA) (*1*). Testing with a panel of single HLA antigen beads (*2*) was crucial in the identification of the epitopes. With the recent development of single antigen C locus beads, it has now become possible to expand investigations into the epitopes for the C locus. Of special interest is that the C locus epitopes are often shared with the A and B locus. In fact, two of the previously described AB locus epitopes have now been determined to also contain C locus specificities.

As noted earlier (*1*), the epitopes explain the previously observed serologic cross-reactivity often described as cross-reactive groups (CREGs) (*3*, *4*). The epitopes, which are based on the amino acid structure of the HLA molecule, underlie the observed cross-reactions of antibodies. They explain why immunization to a single antigen can result in antibodies against a series of antigens. These antibodies, often described as nondonor specific antibodies (NDSA), can now be understood as reactions to HLA antigens sharing the same epitope. The importance of identifying these epitopes is that, according to the humoral theory of transplantation (*5*, *6*), they are the "transplantation antigens" responsible for antibody-mediated transplant rejection.

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MATERIALS AND METHODS

Monoclonal Antibodies and Allosera

Twenty-two mouse mAbs, supernatants or aliquots of ascites, diluted 1:10 to 1:20,000 were used in this study. Because most of the mAbs were not purified, protein concentrations of the final dilutions were not determined. Their specificity has been previously characterized by both serological and immunobinding assays. Because mAbs by definition are monospecific, adsorption experiments were not generally conducted.

Twenty-one anti-HLA alloantibody samples were obtained from multiparous women, placentas, or patients undergoing platelet transfusions or transplants. Sera were adsorbed by an appropriate rHLA SA cell line derived from the LCL712.2 B host cell line. The rHLA single antigen cell line used for adsorption of the antibody was selected based on the known serological specificity of each sample. Forty microliters of serum (diluted 1:3) was mixed with $3–5\times10^6$ cells and subsequently incubated for 30 min at room temperature (RT). The cells were then centrifuged to remove the adsorbed sera for testing. Some sera were adsorbed using a purified rHLA single antigen (SA) attached to microsphere beads. 0.5×10^6 beads were used per adsorption and the antibody was eluted as described below.

Antibody Eluates

After adsorbing the allosera with the cell lines or beads, the cells/beads were washed with phosphate-buffered saline. The adsorbed antibody was then eluted by mixing 60 μl of ImmunoPure IgG Elution Buffer (Pierce, Rockford, IL; catalog 21004) with the cells or beads, and then incubating for 10 min at room temperature. After incubation, the eluates were separated by centrifugation, removed, and neutralized by 3 μl of 1 M TRIS-HCl pH 9.5. Most eluates were from an initial adsorption/elution step. However, in some cases the initial

eluate was adsorbed by another recombinant cell line and the second eluate was tested with the single-antigen beads.

Single Antigen Beads Assays

Monoclonal antibodies, or antibody eluates were tested with 95 HLA class I (A, B, and C-locus) rHLA single antigens individually coupled to different microsphere beads, and with negative and positive control beads (LABScreen beads: LS1A01, LS1A02 and LS1A03, One Lambda Inc. Canoga Park, CA) (*2*). The HLA alleles represented in the SA bead panel are listed in Table 1. LABScreen assays were performed according to the manufacturer's protocol.

Data Analysis

Data generated from the LABScan 100 were analyzed using computer software. Trimmed mean fluorescence values for the SA beads reactions were obtained from the output (.csv) file generated by the flow analyzer and were adjusted for background signal using the formula ([sample #N bead–sample negative control bead]–[negative control #N bead–negative control negative control bead]). The adjusted reaction values were then normalized by multiplying each value by a corresponding normalization factor derived from the results of the mAb W6/32 with the same beads. Normalization factors were calculated by dividing the average value of all mAb W6/32 reactions by the adjusted fluorescence value for each bead. The data were then graphed using either Excel spread sheets or HLA Visual software (One Lambda Inc. Canoga Park, CA). All normalized reactions that were above zero were considered as potential positive reactions for this study, but values for the epitope-specific reactions were rarely below 400.

HLA Amino Acid Sequences, Epitopes, and Distances Between Residues

Amino acid sequences of the HLA antigens or alleles were downloaded from the Anthony Nolan internet website (*7*). Based on the data analysis mentioned above, we determined the positive antigens for each mAb or eluted alloantibody. An epitope search program was then utilized to identify distinguishing amino acids (aa) that are exclusively shared by the positive antigens at particular sequence positions. The program searched for one, two, three, or four common unique aa positions. Among the many possibilities generated, we selected for consideration the positions that are exposed to the surface of the molecule and that are within the antibody binding span estimated at 494Å^2 (19×26Å) (*8*) or 750Å^2 (*9*). Approximate distances in angstroms between two amino acids were calculated using the Cn3D Viewer software (*10*) and the three-dimensional structure of an HLA-A0201 molecule 1QEW (*11*). Amino acids that were exclusively unique to a group of antigens that were reactive with a mAb or an eluted alloantibody, preferably exposed to the surface of the molecule, and within the binding span of the antibody were considered a distinguishing characteristic of the epitope. Epitope ID numbers were assigned depending upon the number of unique aa sites involved (1–200 for one aa, 201–400 for two aa, or 401–600 for three or four aa positions).

RESULTS

A total of 49 HLA class I epitopes were recognized by the series of monoclonal antibodies and eluted alloantibodies used in this study. Four epitopes from our previous study (*1*), where only A and B loci antigens were analyzed, are now

TABLE 1. Single rHLA class I antigens coated on beads used for immunobinding assay

Antigen	Allele	Antigen	Allele	Antigen	Allele	Antigen	Allele	Antigen	Allele
A1	A0101	A36	A3601	B41	B4101	B58	B5801	Cw1	Cw0102
A11	A1101	A43	A4301	B41	B4102	B59	B5901	Cw2	Cw0202
A11	A1102	A66	A6601	B42	B4201	B60	B4001	Cw4	Cw0401
A2	A0201	A66	A6602	B44	B4402	B61	B4002	Cw5	Cw0501
A2	A0203	A68	A6801	B44	B4403	B62	B1501	Cw6	Cw0602
A2	A0206	A68	A6802	B45	B4501	B63	B1516	Cw7	Cw0702
A23	A2301	A69	A6901	B46	B4601	B64	B1401	Cw8	Cw0801
A24	A2402	A74	A7401	B47	B4701	B65	B1402	Cw12	Cw1203
A24	A2403	A80	A8001	B48	B4801	B67	B6701	Cw14	Cw1402
A25	A2501			B49	B4901	B7	B0702	Cw15	Cw1502
A26	A2601	B13	B1301	B50	B5001	B71	B1510	Cw16	Cw1601
A29	A2901	B18	B1801	B51	B5101	B72	B1503	Cw17	Cw1701
A29	A2902	B27	B2705	B51	B5102	B73	B7301	Cw18	Cw1802
A3	A0301	B27	B2708	B52	B5201	B75	B1502	Cw9	Cw0303
A30	A3001	B35	B3501	B53	B5301	B76	B1512	Cw10	Cw0302
A31	A3101	B37	B3701	B54	B5401	B77	B1513	Cw10	Cw0304
A32	A3201	B38	B3801	B55	B5502	B78	B7801		
A33	A3301	B39	B3901	B56	B5601	B8	B0801		
A33	A3303	B39	B3905	B57	B5701	B81	B8101		
A34	A3401	B4005	B4005	B57	B5703	B82	B8201		

TABLE 2. HLA class I (A and B) epitopes recognized by a series of anti-HLA monoclonal antibodies or allosera

Antibody/serum tested	mAb or Allo	rHLA cells used for adsorption[a]	Single antigen beads with positive reactions[a]	Epitope number assigned[b]	Position and unique amino acid for possible epitope sites[c]
Z5550.DM	M	N/A	B46	10	[69R][d]/46A+66K
X7768.TO	M	N/A	B8	11	[9D][d]/(67F)+131R/(67F)+177D/(67F)+180E
Z5571.I0	M	N/A	A23, 24, 80	28	62E
F2164-4A7A3	M	N/A	A32, 74, **B7**, 8, 13, 18, 27, 35, 37, 38, 39, 4005, 41, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 67, 71, 72, 73, 75, 76, 77, 78, 81, 82	33	[109L][d]
FN3814-3E1G4	M	N/A	B18, 35, 37, 51, 52, 53, 78, 58	35	45T
F352-10F9E1	M	N/A	A25, 32, **B7**, 8, 13, 18, 27, 35, 37, 38, 39, 4005, 41, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 67, 71, 72, 73, 75, 76, 77, 78, 81, 82	233	[79R +127N][d]
X7021.E0	M	N/A	B27, 47, 61, 7, 48, 60, 73, 81, 13	235	138T+163E
Z1173.N0/F667-2E1E4/ 2005-01035(Allo)	M	N/A	B57, 58	236	43P+62G/ 41A+43P+62G/17R+41A+43P+62G/ 19E+41A+43P+62G
X8442.E0	M	N/A	B57, 63	237	46A+65R/41A+46A+65R "B58 is weak positive (41A+46E+65R)"
FG2755-11A3D7	M	N/A	A1, 2, 3, 11, 25, 26, 29, 32, 33, 34, 36, 43, 66, 68, 69, 74, **B57,** 58, 63	238	56G+65R
0544HA	M	N/A	A1, 2, 3, 1101, 26, 29, 30, 31, 33, 34, 36, 43, 66, 68, 69, 74, 80	242	19E+79G
FC1043-6B4D1B9	M	A2501	A25, 26, A33, 34, 66, 68, 69, **B63**	243	62R+65R "B76 also positive (62R+65Q)"
X4221.DQ/ X3494.N0	M	N/A	B2705	406	65Q+69A+ 80T 65Q+69A+82L 65Q+69A+83R
X8598.T0/S8036	M	N/A	A24	407	127K+142I+144K/ 127K+142I+151H/ 127K+144K+145R/ 127K+145R+151H
Z5754.R0	M	N/A	B7	408	(147W)+163E+177D (147W)+163E+180E
Z6856.PK	M	N/A	B63	409	43P+62R+65R
F633-3E8H4	M	N/A	B46, 57, 58, 63	415	[(63E)+(71A)+163L][d]
F698-4F9F9	M	N/A	A11, **B57,** 58	417	[(9Y)+41A+(63E)+ (95I)][d]
X6954.TO	M	N/A	A26, **B13**	418	(32Q)+62R+ (77N)+80T (45M)+62R+ (77N)+80T
FC2121-5A4A1	M	N/A	B49, 51, 52, 63, 77	419	80I+90A+127N+(152E)/ 80I+109L+131S+(152E)/ 82L+90A+127N+(152E)/83R+90A+127N+(152E)
FS964-1A3B4	M	N/A	B8, B64, B65	420	69T+(74D)+158A+163T
S33S	A	nn	A203, 25, 26, 34, 43, 66	27	149T
2004-08361	A	nn	A80	29	56E/62E+65R/ 62E+76A/ 144K+151R/163E+166D/ 163E+167G
2002-05676/2002-00048	A	nn	A1102	30	19K

TABLE 2. Continued

Antibody/serum tested	mAb or Allo	rHLA cells used for adsorption[a]	Single antigen beads with positive reactions[a]	Epitope number assigned[b]	Position and unique amino acid for possible epitope sites[c]
2002-00142	A	nn	B46, 73	239	43P+76V/ 65Q+76V/ 76V+79R/ [76V+80N 41A+43P+76V/ 41A+65Q+76V/ 73T+76V+79R/ 73T+76V+80N][d]
2002-01731	A	nn	A30, 31	31	56R
2005-02583	A	nn	A30	36	17S/56R+73T
S35C	A	N/A	B7, 48, 81	231	41A+178K
AS627	A	N/A	B7, 42, 46, 54, 55, 56, 67, 81, 82	234	[43P+(70Q)/65Q+(70Q)][d]
0-4/38991/ 38995/ 38998/ 39002	A	nn	B76	240	163L+166D/ 163L+167G
AS264	A	A3601	A1, 11, 25, 26, 34, 36, 43, 6601, 80	241	65R+90D/43Q+90D/90D+138M
2002-00323	A	nn	A11	404	149A+150A+163R/ 149A+158A+163R/ 149A+163R+166E/ 149A+163R+167W
2002-05252	A	nn	B7, 42, 54, 55, 56, 67, 81, 82	410	[41A+46E+67Y/43P+46E+67Y/43P+46E+70Q][d]/ 43P+69A+70Q/43P+70Q+76E/[46E+65Q+67Y/ 46E+65Q+70Q][d]
2005-04739	A	nn	B2708	411	(63E)+69A+80N/(70K)+76E+80N/(70K)+76E+ 82R/(70K)+76E+83G/(70K)+80N+131S
2002-00245/2004-08903	A	nn	A0201	412	(9F)+142T+149A/(9F)+145H+149A
2002-01799	A	nn	B49, 52, 63	414	62R+(63E)+80I

[a] Alleles are designated only when other alleles of the same antigen did not react with the antibody.

[b] Epitopes 27, 28, 30, 31, 33, 35, and 36 can be defined by a single amino acid at a unique position on the HLA molecule. Epitope 29 can be identified by either one or 2 aa combined. Epitopes 10, 11, and 231–243 involve two amino acids/positions considered in combination, with the exception of epitopes 236, 237, and 239, which can also be defined by 3 or 4 aa alternatives. Epitopes 404–420 can be identified by three or more amino acids.

[c] Possible alternative epitope definitions are separated by a slash. Epitopes that are defined by more than a single position/aa are separated by a plus sign. Amino acids that are not exposed at the surface of the HLA molecule are in parentheses.

[d] Epitope also shared by C-locus antigens based on aa sequences but not proven by antibody test with SA beads are in square brackets.

aa, amino acids; nn, absorption and elution were not needed; N/A, not applicable.

redefined (epitope 10 and 11) or the C-locus antigens are included (epitopes 205 and 222). For example, epitope 10 (Table 2) was earlier defined by the aa arginine (R) at position 69. However, when aa sequences for the C-locus were included in the analysis, all C-locus antigens were found to have the aa arginine at this position; therefore 69(R) is not exclusively unique to the B46 antigen. However, the amino acids alanine (A) and lysine (K) at positions 46 and 66 respectively are uniquely exclusive to B46 and therefore they identify the epitope. Epitope 222 (Table 3) is now shown to be shared by the C-locus antigens (Cw2 and Cw17) in addition to A6602, B7, B13, B27, B47, B48, B60, B61, B73, and B81 antigens.

Overall, we found 45 new epitopes which include 11 shared by A locus antigens, 17 shared by B locus antigens, 4 shared by C locus, and 13 interlocus epitopes of which 6 shared by A and B, 5 shared by B and C, and 2 shared by A, B, and C antigens.

The mAbs selected for this study recognized epitopes with one to four unique aa at certain position(s) (Tables 2 and 3). The combination aa positions were not contiguous but

TABLE 3. HLA A, B, and C loci class I epitopes recognized by a series of anti-HLA monoclonal antibodies and allosera

Antibody/serum tested	mAb or Allo	rHLA[a] cells used for adsorption	Single antigen[a] beads with positive reactions	Epitope number assigned[b]	Position and unique aa for possible epitope[c] sites
FS32-5D8B6	M	N/A	**A2**, 3, 11, 2403, 25, 26, 29, 30, 31, 32, 33, 34, 36, 43, 66, 68 , 69, 74, **B7**, 8, 13, 18, 27, 35, 37, 38, 39, 4005, 41, 42, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 67, 71, 72, 73, 75, 77, 78, 81, **Cw1**, 2, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15, 16, 17, 18	32	167W
F1119-9F4E7	M	N/A	**A32**, 74, **B7**, 8, 4005, 41, 42, 48, 60, 61, 73, 81, **Cw1**, 2, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15, 16, 17, 18	205	109L+131R
Z2611.R0	M	N/A	**B54**, 55, 59, **Cw1**, 4, 5, 6, 7, 8, 12, 14, 15, 16, 18	232	(103L)+163T
A132	A	Cw0702	Cw7	37	194L
A39/A40	A	A2501/A0201	**A2**, A25, A26, A29, A31, A32, A33, A34, A43, A66, A68, A69, A74, **B73**, **Cw7**, Cw17	38	253Q
Z7921.00/A113	A	Cw0202/Cw0303 (Cw9)	Cw2, 9, 10, 15	39	21H
A129	A	nn	Cw5, 8	40	177K
A61	A	Cw1701	B73, **Cw7**, 17	41	267Q
A4	A	Cw0202	A6602, **B7**, 13, 27, 47, 48, 60, 61, 73, 81, **Cw2**, Cw17	222	163E+166E/ 163E+167W
A6	A	Cw0102	B46, **Cw1**, 8, 9, 10, 14, 16	421	(73T), 76V, 80N, 90A
A113	A	Cw1701	Cw2, 4, 5, 6, 15, 17, 18	244	77N+80K
A6	A	B62 (B1501)/B35	**B35**, 4005, 46, 49, 50, 51, 52, 53, 56, 57, 58, 62, 63, 71, 72, 75, 77, 78, **Cw9**, Cw10	245	163L+167W
Z9016.00/ 2002-00142	A	Cw1802/ nn	**B46**, 73, **Cw1**, 7, 8, 9, 10, 12, 14, 16	246	76V+80N/ 73T+76V+ 79R

[a] Alleles are designated only when other alleles of the same antigen did not react with the antibody.

[b] Epitopes # 32 and 37–41 can be defined by a single amino acid at a unique position on the HLA molecule. Epitopes 205, 232 and 222–246 depend on either two, three, or four amino acids/positions considered in combination.

[c] Possible alternative epitope definitions are separated by a slash. Epitopes that are defined by more than a single position/aa are separated by a plus sign. Amino acids that are not exposed at the surface of the HLA molecule are in parentheses.

aa, amino acids; nn, absorption and elution were not needed; N/A, not applicable.

Bold indicates the beginning of a different locus.

were within a conformational distance allowing antibody binding. The interlocus epitope 238 (Table 2) is an example of an epitope defined by two uniquely shared aa positions. The reactive HLA antigens A1, A2, A3, A11, A25, A26, A29, A32, A33, A34, A36, A43, A66, A68, A69, A74, B57, B58, and B63 were all found to have glycine (G) at position 56 and arginine (R) at position 65. Although several other HLA class I antigens have 56(G) and 65(R), none of these antigens share both amino acids at these two positions, making the aa combination unique for this mAb-reactive group of HLA antigens.

For the allosera, eluates recovered from the rHLA SA cell lines were found to recognize epitopes consisting of unique aa residues at 1, 2, 3, or 4 sequence positions (Tables 2 and 3). Epitope 241 in Table 2 is an example of a two aa epitope that was defined by serum AS264 adsorbed with a recombinant cell line expressing only the HLA-A3601 antigen. The eluted antibody reacted with A1, A11, A25, A26, A34, A36, A43, A6601, and A80 antigens. All nine antigens share one epitope defined by any of the three two-aa combinations listed. For example, the combination of the two amino acids arginine (R) at position 65 and aspartic acid (D) at position 90, located in the alpha-1 domain of the HLA alpha molecule, can define this epitope.

Absorptions and elutions were not needed (nn) for most of the allosera listed in Table 2. However, most allosera that defined epitopes present on C-locus antigens were absorbed by rHLA single antigen cell lines and the eluted antibody was tested with the A, B, and C SA beads (Table 3).

The actual fluorescence data for several of the immunobinding reactions are presented graphically (Figs. 1–3) for the positive (epitope-specific) reactions for three allosera. Only the highest negative reactions are included to show the marked drop in signal from the epitope positive antigens; the other SA beads had little or no fluorescent signal. The figures also depict the relevant aa residues at the pivotal antibody-binding positions, as defined by their unique sequence composition for the reactive HLA alleles on the SA beads.

Figure 1 shows the results of the first eluate, from the B3501 rHLA cell line, illustrating an epitope that is shared by three C and 20 B locus antigens. Cw9, Cw0302 (Cw10), and Cw0304 (Cw10), B4005, B46, B63, B53, B49, B75, B35, B62, B77, B50, B56, B71, B5703, B52, B5701, B78, B72, B5102, B58, and B5101 all share the amino acids leucine (L) and tryptophan (W) at positions 163 and 167, respectively. Combined, these amino acids, which are exclusive to these antigens at the two positions, define the epitope (no. 245, Table 3).

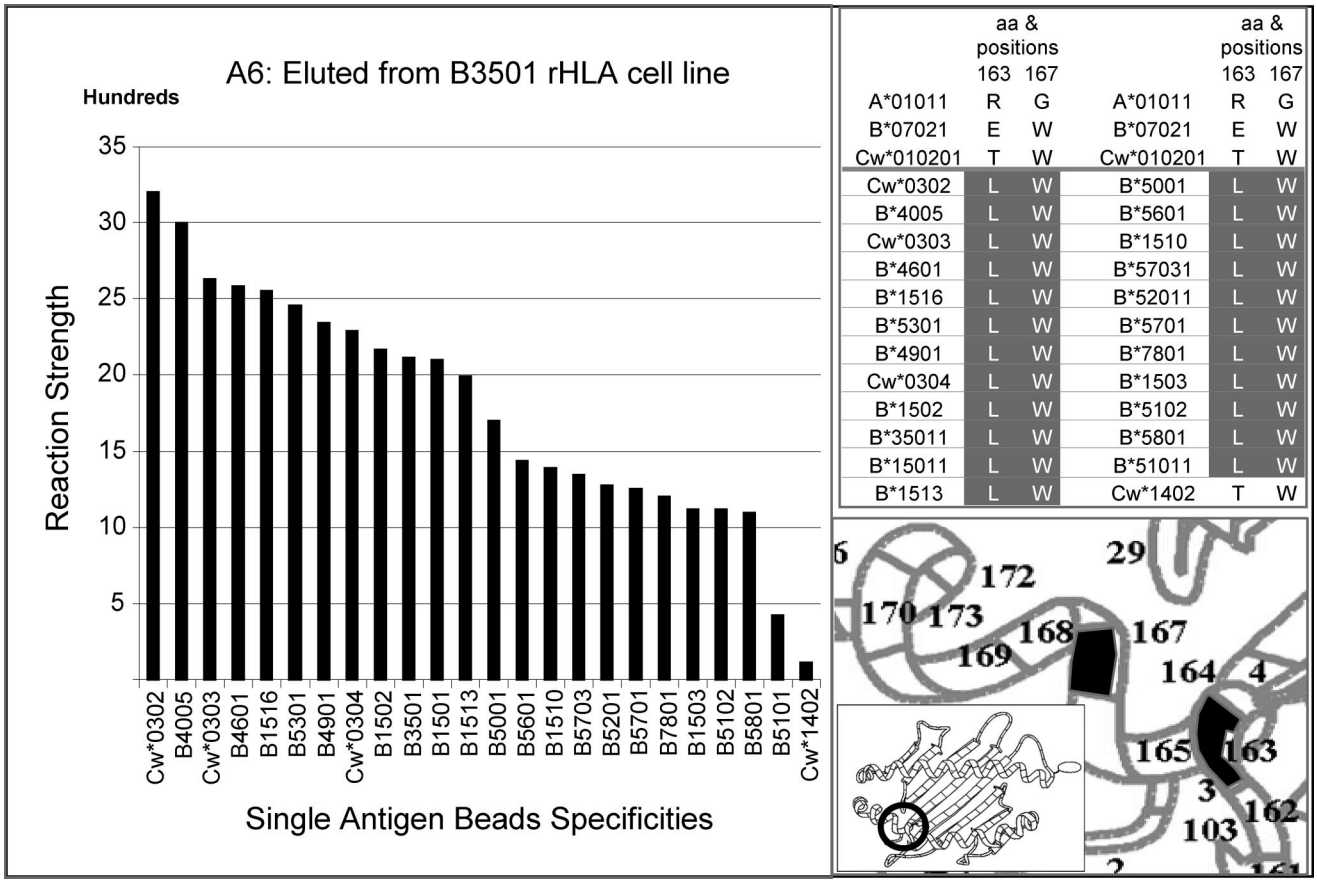

FIGURE 1. Alloantibody eluted from the rHLA SA cell line B3501 shows strong reactions with the C locus antigens Cw9, Cw0302 (Cw10), and Cw0304 (Cw10) and the B-locus antigens B4005, B46, B63, B53, B49, B75, B35, B62, B77, B50, B56, B71, B5703, B52, B5701, B78, B72, B5102, B58, and B5101 which all share the amino acid leucine (L) at position 163 and tryptophan (W) at position 167. The two amino acids in combination define a common epitope shared by the positive antigens (epitope 245, Table 3).

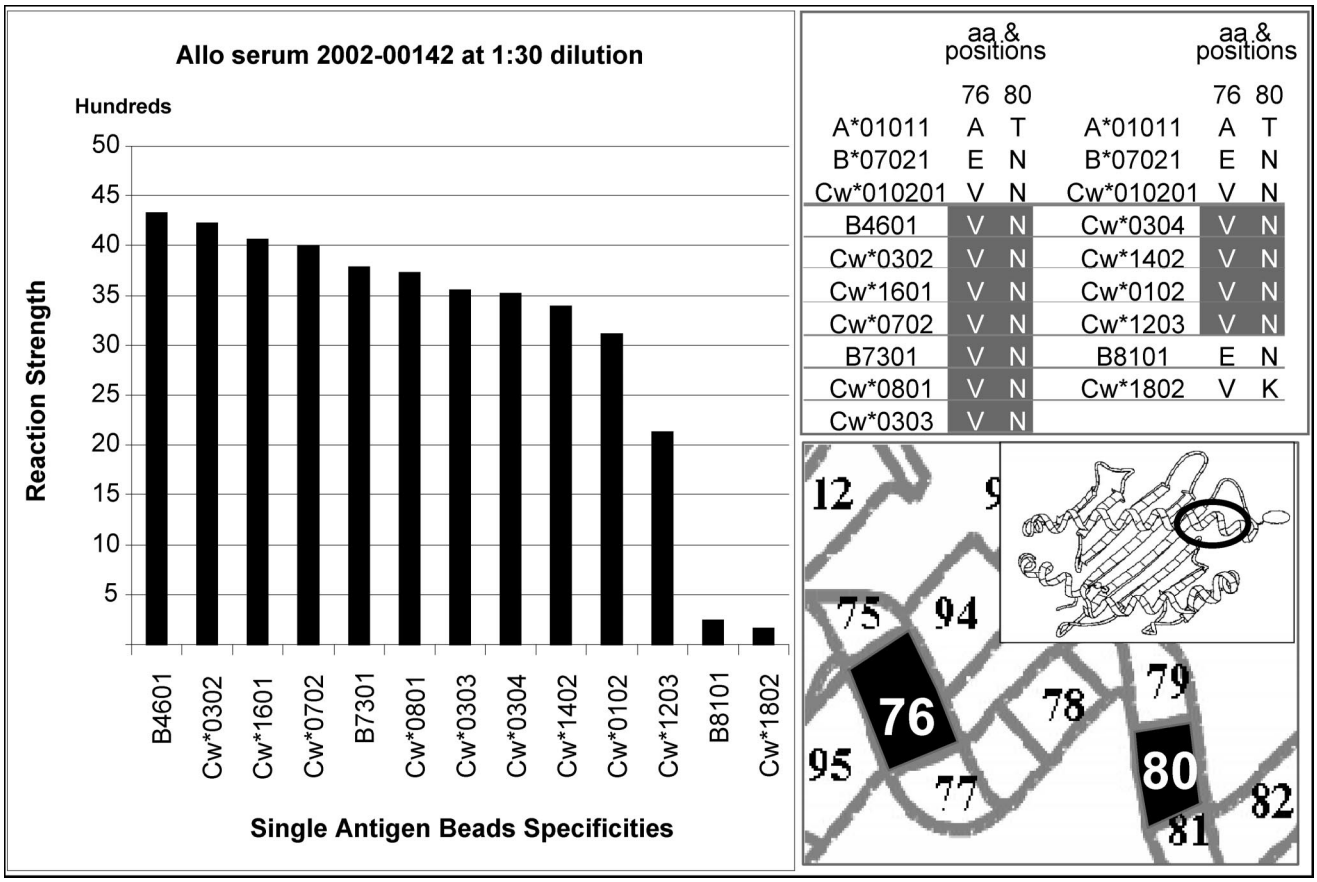

FIGURE 2. Example of an alloserum positive with B and C antigens all sharing a unique epitope. Antigens B46, B73, and Cw0302 (Cw10), Cw1601, Cw0702, Cw0801, Cw0303 (Cw9), Cw0304 (Cw10), Cw1402, Cw0102, and Cw1203 share the amino acid valine (V) at position 76 and asparagine (N) at position 80. Both amino acids are located in the alpha 1 domain of the HLA antigen and combined define the epitope (epitope 246, Table 3).

Figure 2 illustrates the SA beads reactions to the B46, B73, and Cw0302 (Cw10), Cw1601, Cw0702, Cw0801, Cw0303 (Cw9), Cw0304 (Cw10), Cw1402, Cw0102, and Cw1203 antigens that share the aa valine (V) at position 76 and asparagine (N) at position 80. Both amino acids are located in the alpha 1 domain of the HLA antigen and combined define the epitope (no. 246, Table 3).

Figure 3 shows an example of an epitope shared by A, B, and C class I antigens. Epitope 38 is shared by 19 A locus, one B locus, and two C locus antigens. Antigens A3303, A6801, Cw0702, Cw1701, A7401, A3301, A6901, A6802, A3401, A2901, A2902, A2501, A0203, A2601, A3101, A3201, A0201, A6601, A4301, A6602, A0206, and B7301 all share the aa glutamine (Q) at position 253. Position 253 is located in close proximity of the cellular membrane on the alpha chain of the HLA antigen. Although the aa is exposed to the surface of the molecule, it is uncertain that it is accessible for antibody binding due to its location. However, glutamine is the only aa we found that is exclusively common to all positive antigens, listed above, and may therefore play a role in defining this epitope (no. 38, Table 3).

DISCUSSION

In this study, we used recombinant single antigens to determine the specificities of every serum and monoclonal antibody tested. All recombinant single antigens were developed in a mammalian cell expression system of HLA transfected cells. This system provides all of the crucial elements, including posttranslational modification, to produce mature glycosylated HLA antigens that are indistinguishable from native HLA class I antigens (*2*).

We have described earlier (*1*) the unique ability of class-I A and B SA beads to identify 58 HLA class I epitopes recognized by mAbs and alloantibodies. Here we add 34 more epitopes shared by A, B, or A and B antigens (Tables 1 and 2). Thus, in combination with the previously published table, there are now 92 A and B locus epitopes. We estimate that these epitopes will be the common ones encountered in the American population. The total permutations of amino acid combinations which can make up epitopes are immense, but in practice, we can hope that the eventual number that will function as immunogenic epitopes will be rather limited.

We concentrated here on finding the epitopes of the C-locus antigens. We describe 13 epitopes which were either found exclusively on C locus or shared with other antigens. Two epitopes which we had described earlier as AB epitopes when tested with new C locus single antigen beads were found here to also contain C locus antigens. Epitopes 205 and 222 (Table 3) identified by a mAb and an alloantibody eluted from Cw0202 rHLA SA cell line respectively, when retested

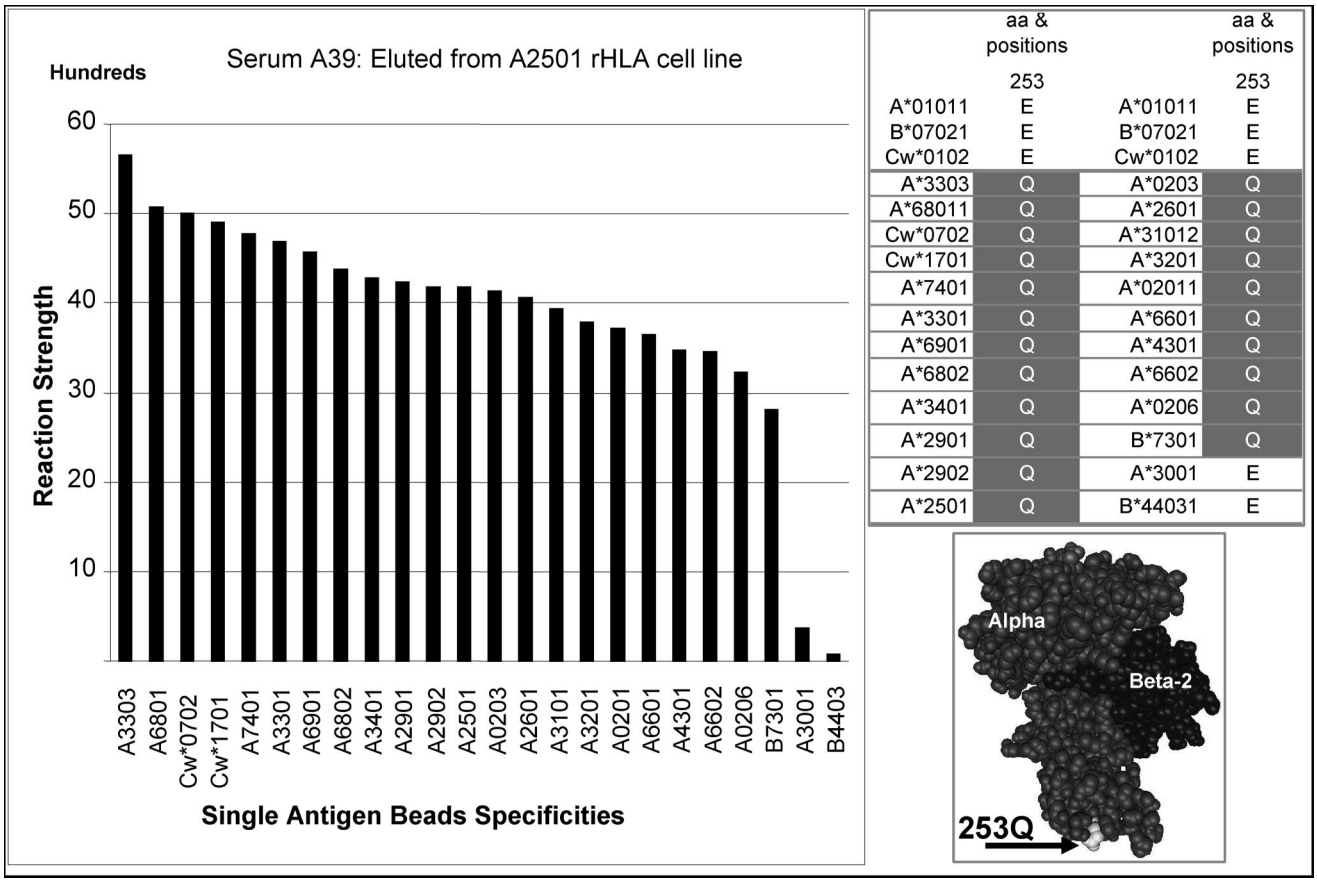

FIGURE 3. Example of an interlocus epitope shared by A, B, and C class I antigens. Antigens A3303, A6801, Cw0702, Cw1701, A7401, A3301, A6901, A6802, A3401, A2901, A2902, A2501, A0203, A2601, A3101, A3201, A0201, A6601, A4301, A6602, A0206, and B7301 shared the amino acid glutamine (Q) at position 253. Although glutamine at this position is located close to the cell membrane and may not be easily accessible to the antibody, it is the only amino acid exclusively shared by the positive antigens (epitope 38, Table 3).

with A, B, and C loci SA beads; all C locus antigens sharing the same amino acids with the A and B antigens were also positive.

For most A, B, and C antigens, the epitope is often located in the alpha 1 and alpha 2 domains on the alpha chain of the HLA antigen. We note here that three epitopes (37, 38, 41) are identified by a single amino acid at positions located in the alpha 3 domain, and in the case of epitope 38 (253Q) the amino acid is in close proximity to the cell membrane. It is therefore uncertain, at least for epitope 38, whether it is accessible to bind the antibody. However, it is the only amino acid that is unique to the group of antigens sharing epitope 38, and the role that 253Q may play in defining the epitope can not be determined in this study. Several epitopes were observed more frequently than others epitopes among the list of 13 C locus defined. In particular, epitopes 38, 222, and 244 were found in several other sera.

The cell surface expression of the C locus antigens has been estimated at 10% that of the A and B antigens (*12*). Payne et al. studied the reactivity of the B46 antigen with alloantisera specific to the Cw1 and Cw3 HLA antigens (*13*). The molecular basis of reactivity of anti Cw1 and anti Cw3 alloantisera with the HLA-B46 haplotypes was reported by Zemmour et al. (*12*, *14*). We note here that B46, Cw1, and Cw3 also share epitopes 32, 246, and 421 with other antigens (Table 3). For example, epitope 246 is shared by Cw7, Cw8, Cw12, Cw14, Cw16, and B73 in addition to B46, Cw1, Cw9 (Cw3), and Cw10 (Cw3). The expansion to all of the antigens sharing one epitope was made possible by the use of the SA beads.

Most of the alloantibodies in this study are from pregnancies. However, sera from transplant patients, recently tested, were also found to have C-locus specificities (data not shown). The epitopes identified by the antibodies were probably the targets of antibody attack (*6*). According to the humoral theory (*5*, *6*), these epitopes can also be the key factors that need to be matched for transplants as was noted earlier (*15*). Matching for the C locus antigens, as advocated for bone marrow transplants, may be indicated for organ transplants. The relative strengths of the epitopes as immunogenicity will be of importance in selecting mismatches for unrelated bone marrow transplants when a complete match cannot be found.

Duquesnoy has proposed triplet epitopes taking three amino acids around variable positions on the HLA molecule (*16*) and recently proposed "patches" of amino acids within 3.0 to 3.5 angstroms of a variable position where one or two patches (epilets) identify epitopes that are conformational in nature (*17*). In contrast to these theoretic epitopes, the

epitopes described here are based on actual antibody reactions identified by SA beads. In many instances, several antibodies were found which produced identical reactions. Hopefully, others will also find antibodies that react to the exact epitopes described here.

Epitopes identified here explain many of the complex antibodies previously found in the sera of multiparous women, multitransfused patients, and patients who had rejected an organ transplant. We have previously called attention to the difficulty in finding all the antibodies in a serum by conventional methods, and the need for testing with SA technology (*18*). The complex series of antibodies identified by the SA beads can be best understood when they are defined by the epitopes actually recognized. Screening for the presence of donor-specific antibodies in solid organ transplant patients often reveals that even a single "antigen" mismatch can result in the production of antibodies to many other "antigens." This phenomenon can now be understood when considering the epitope that was mismatched and the corresponding immune response to the epitope. Thus, rather than describing the series of antigens reacting with an antibody, we propose to assign the antibody specificity to the target epitope(s).

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