



HLA class II DQA and DQB epitopes: Recognition of the likely binding sites of HLA-DQ alloantibodies eluted from recombinant HLA-DQ single antigen cell lines



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ABSTRACT

Donor-specific antibodies (DSA) in sera of sensitized transplant patients are often produced against the specific epitopes on mismatched HLA antigens. In this study, we selected sera from 30 kidney transplant patients with DSA and AMR to define DQ epitopes. Using adsorption and elution assays, we identified 18 antibody reaction patterns to define 6 new epitopes and to confirm 12 previously defined epitopes. In one patient case, one mismatched antigen produced 3 different antibodies and, in another, antibodies were produced against the alpha and beta chains of the same antigen. For some sera, a single epitope can explain reactions for 27 of the 29 DQ beads in the single antigen panel.

Several studies highlighted the prevalence of anti-DQ antibodies. In 2011, Almeshari et al. observed DQ DSA in 34/46 (74%) of rejection episodes – 44 patients had DSA and 20 lost their graft due to AMR. Other studies have shown a high prevalence of anti-DQ antibodies and an association with adverse effects on the graft.

We conclude that analysis of the epitopes of the DQ antibodies using Adsorption/Elution and testing on single antigen DQ beads helps to better understand the specificities and cross-reactions of DQ antibodies in transplant patients.

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1. Introduction

Donor-specific antibodies (DSA) in sera of sensitized transplant patients are often produced against the specific epitopes on mismatched HLA antigens. In 2008, we defined HLA-DQ epitopes using mouse monoclonal and alloantibodies adsorbed and eluted (Adsorption/Elution) from HLA-DQ homozygous B-lymphoblast cell lines [1]. In this study, we selected sera from kidney transplant patients with antibody-mediated rejection (AMR) and DSA to define DQ epitopes. Here, we used recombinant HLA-DQ single antigen cell lines to adsorb and elute the antibodies. We confirmed

twelve of the 18 previously defined epitopes and six new epitopes are added.

Our interest in defining new DQ epitopes and identifying the DQ alleles that share these epitopes was to advance our knowledge of DQ antibody reactivity. DQ antibodies are now getting more attention because they are found at high frequency in the sera of transplant patients. Previously, Almeshari et al. observed that DQ DSA were present in 34/46 (74%) of rejection episodes, in which 44 patients developed antibody-mediated rejection (AMR) and DSA, with 20 patients losing their graft due to AMR [2,3]. Several other studies reported anti-DQ antibodies-as high as 80% in one study [4–6]. Willicombe et al. reported 18.2% anti-DQ antibodies of which 54.3% were *de novo* DSA and showed associations with a significant risk of AMR, transplant glomerulopathy (TG), and allograft loss [7]. Worthington et al. reported DQ antibodies in patients are strongly predictive of transplant failure [8]. DeVos et al. noted that DQ DSA were the most common class II antibodies and that may contribute to inferior graft outcomes [9].

Although only six new epitopes were added and 12 were confirmed, we believe that the recombinant single antigen HLA-DQ

Abbreviations: SA, single antigen; Adsorption/Elution, adsorbed and eluted; AMR, antibody-mediated rejection; TG, transplant glomerulopathy; DSA, donor-specific antibody; rHLA, recombinant HLA cell line; PBS, phosphate buffered saline; RT, room temperature, MFI, mean fluorescence intensity; DQA, DQ alpha chain; DQB, DQ beta chain.

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cell lines are a reliable tool for adsorbing and eluting monoclonal alloantibodies. By doing this type of adsorption and elution, we identified and characterized different antibodies produced against mismatched alleles, defined the epitopes they target, and identified the alleles that share the epitope.

2. Materials and methods

2.1. Allosera

Sera from 30 kidney transplant patients, who had undergone antibody mediated rejection (AMR), were selected because they developed donor-specific anti-HLA DQ antibodies.

2.2. Adsorption and elution

For adsorption and elution (Adsorption/Elution) of antibodies, we used 15 different recombinant HLA (rHLA) DQ cell lines, derived from the K562 cell line, each expressing a single DQ heterodimer. These lines are denoted with an RC in the cell line name. We also used one homozygous cell line, TER124. Alloserum, diluted 1:3 in 1X phosphate-buffered saline (PBS), was mixed with 1.5–3 million rHLA cells and incubated for 30 min at room temperature (RT). The cell suspension was then centrifuged, supernatant removed, and the cell pellet was washed twice with 1× PBS to remove any residual, unbound antibodies. Adsorbed antibodies were then eluted by adding 60 µl of ImmunoPure IgG Elution Buffer (Pierce, Rockford, IL; catalog #21004). After 10 min incubation at RT, the eluates were separated from the cells by centrifugation and neutralized with 3 µl of 1 M TRIS–HCl (pH 9.5). In some cases, to clarify the reaction pattern, two subsequent adsorption/elutions were done with different cell lines in which the eluate from the first Adsorption/Elution was then adsorbed and eluted from a different cell line in the second.

2.3. Antibody detection

Allosera (diluted 1:3 and diluted 1:10) and eluates were all tested for HLA class II antibodies by multiplex bead assay, which contains negative control bead, positive control bead and 91 unique microspheres each coupled to a different rHLA Class II antigen (LABScreen® Class II Single Antigen (SA), Catalog #LS2A01 lot #99). LABScreen® assays were performed according to the manufacturer's protocol. Briefly, allosera were incubated with beads for 30 min at RT. The beads were then washed 3 times with wash buffer, incubated with a secondary antibody (Goat anti-Human IgG PE) for 30 min at RT, washed 2 times with wash buffer, re-suspended in 1× PBS, and read on the Luminex LABScan 100 flow cytometer (One Lambda Inc., now a part of ThermoFisher Scientific). With every assay, we ran a positive control serum, negative control serum, and neutralized elution buffer to determine the background due to non-specific binding.

2.4. Data analysis

Trimmed mean fluorescence intensity (MFI) values for bead reactions, obtained from the output (.csv) file, were adjusted for background using the following formula: [(sample #N bead) – (sample negative control bead)] – [(negative serum control #N bead) – (negative serum control negative control bead)]. Normalized values were used to generate graphs by Microsoft Excel. Generally, normalized reactions above 1000 MFI were considered positive, although in some cases, MFI values between 670 and 1000 were considered positive because the overall reactions of the eluted antibodies were of weak to moderate strength.

2.5. Determination of epitopes

Amino acid sequences of HLA-DQ alpha (DQA) and DQ beta (DQB) chains were downloaded from the Anthony Nolan internet

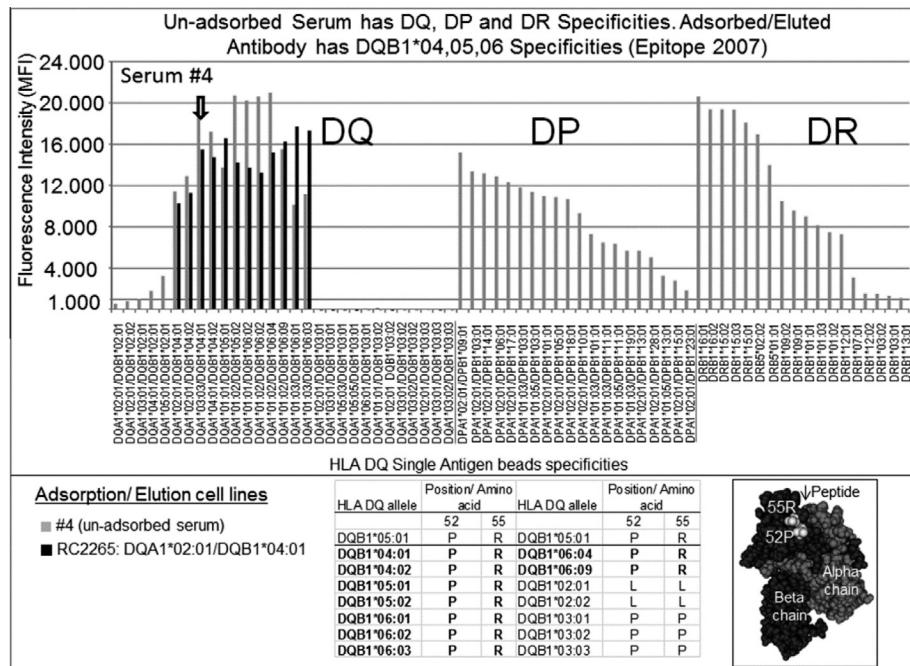


Fig. 1. Serum #4, un-adsorbed, has reactions against DQ, DP, and DR specificities. However, the antibody eluted from rHLA SA cell line RC2265, which expresses heterodimer DQA1*02:01/DQB1*04:01, shows strong reactions against DQB1*04, 05, 06 and is completely negative against DP, DR and other DQ specificities. The epitope exclusively shared by the positive antigens DQB1*04, 05, 06 is defined by a combination of two amino acids, proline (P) at position 52 and arginine (R) at position 55 on the beta chain. The arrow shows the bead with the same heterodimer as the rHLA cell line.

website [10]. Using results from adsorption and elution, an epitope search program was used to identify one, two, or three amino acid(s) that are exclusively shared by antigens identified by eluted antibody positive reactions. Among the possible exclusively shared amino acids, we considered only positions exposed on the antigen surface and that are within the antibody binding span estimated at 494 Å (19×26 Å) or 750 Å [11,12]. Distances between two amino acids were approximated using the Cn3D Viewer software [13] and the three-dimensional structure of HLA-DQ2 heterodimer [14]. We assigned each epitope a provisional numerical # following the format we used in previous studies [1,15].

3. Results

The 30 sera used in this study, each from a different patient, all contained anti-DQ DSA. Most sera had either DR and/or DP specificities as well. Twenty-six sera were adsorbed with one to eight different rHLA cell lines resulting in 77 eluted antibodies plus four non-adsorbed sera, all of which can be categorized into 18 distinct reaction patterns. Each of the eighteen reaction patterns describes a distinct epitope-2 epitopes on the DQA chain and 16 epitopes on the DQB chain.

We defined 6 new HLA DQB epitopes (#2022–2027) listed at the top of Table 2A and confirmed 12 of the 18 previously defined [1]. HLA-DQ epitopes were defined using antibodies that were adsorbed to and eluted from rHLA-DQ single antigen cell lines then tested with Luminex Class II Single Antigen beads.

Adsorption and elution assays are very effective tools to separate an antibody and identify the antigens/alleles which share the epitope it recognizes. Fig. 1 shows the results of un-adsorbed serum #4 which has reactions against DQ, DP, and DR specificities (gray bars). However, the antibody eluted from rHLA SA cell line RC2265, which expresses heterodimer DQA1*02:01/DQB1*04:01, shows strong reactions against DQB1*04, 05, 06 chains and is com-

pletely negative against DP, DR and other DQ antigens. The epitope (2007, Table 2B) exclusively shared by the positive alleles DQB1*04,05,06 (black bars) is defined by a combination of two amino acids, proline (P) at position 52 and arginine (R) at position 55 on the beta chain.

Tables 1 and 2 summarize all 81 different assays performed using the 30 sera. Grouped by epitope, we list the serum used, cell line information, reaction pattern with SA beads, and the minimum and maximum MFI for the reactions. Reaction patterns are denoted by the shaded squares. For example, Adsorption/Elution 1 in Table 1 shows the results for serum #18 which was adsorbed with rHLA-DQ cell line RC2261 (DQA1*02:01/DQB1*03:01) and the eluted antibody reacted with beads 28, 31, 32, 98, 40, 82, and 45 (shaded squares). Here, bead 40 was coated with the same DQ heterodimer as the cell line used for the adsorption elution (DQA1*02:01/DQB1*03:01) and is denoted by AE. Similarly, Adsorption/Elution 2 shows adsorption and elution of the same serum with a different cell line which has the same alpha but different beta chain RC2262 (DQA1*02:01/DQB1*03:02) and shows the same results as Adsorption/Elution 1.

Tables 3 and 4 show the DQA1 and DQB1 typing for the recipient and donor and the mismatched allele for each patient tested. The amino acid substitutions for each recipient at the positions that define the epitope are listed under column "Recipient aa at epitope position". It is clearly shown that in all cases, except in one (Adsorption/Elution #70), that the antibodies are not targeting self-epitopes. For example, Adsorption/Elution #1, the epitope on the mismatched allele DQA1*02:01 is defined by 52H and the corresponding amino acids on the recipient's alleles DQA1*01:02 and DQA1*05:05/09 for position 52 are serine (S) and arginine (R) respectively. In Table 1, Adsorption/Elution #1, we see that all heterodimers with the DQA1*02:01 are positive and those with other DQA alleles are negative including the recipient's alleles – beads 36, 38, 73, 74 and 67.

Table 1

The results for DQA1 epitopes detected by eluted antibodies from the sera of 30 kidney transplant patients. The first column (Adsorption/Elution #) designate the assay # producing the results listed to the right followed by serum ID and the epitope #. The shaded squares indicate all heterodimers that were positive for each eluted antibody and the minimum and maximum MFI values are listed to the right (MFI Min, MFI Max). The cell ID and DQA1 and DQB1 chains of the rHLA DQ cell lines used are shown on the far right (#, DQA1*, DQB1*).

HLA DQA1 Epitopes																										
HLA-DQ heterodimers on SA beads reactive with eluted antibody (Shaded squares show DQA1 chains sharing epitope)																										
Lot 9 Bead			28	31	32	39	70	74	76	77	78	79	80	83												
Antigen			DQ2	DQ2	DQ2	DQ2	DQ4																			
DQB1			*02:01	*02:01	*02:01	*02:01	*04:01	*04:02	*04:01	*04:02	*04:01	*04:02	*04:01	*04:02												
DQA1			*03:01	*03:01	*03:01	*03:01	*03:01	*03:01	*03:01	*03:01	*03:01	*03:01	*03:01	*03:01												
Adsorption/ Elution #	Serum	Epitope #																								
1	18	2017																								
2	18	2017																								
3	18	2017																								
4	22	2017																								
5	22	2017																								
6	22	2017																								
7	33	2017																								
8	33	2017																								
9	35	2017																								
10	35	2017																								
11	2	2018																								
12	11	2018																								
13	13	2018																								
14	13	2018																								
15	13	2018																								
16	21	2018																								
AE Cell lines																										
RC DQA1 DQB1																										
3,932 7,343 2261 *02:01 *03:01																										
3,821 6,647 2262 *02:01 *03:02																										
4,528 7,946 2265 *02:01 *04:01																										
6,580 12,179 2261 *02:01 *03:01																										
7,297 12,887 2262 *02:01 *03:02																										
6,674 12,149 2265 *02:01 *04:01																										
13,170 20,766 2262 *02:01 *03:02																										
12,792 20,832 2261 *02:01 *03:01																										
13,211 20,444 2261 *02:01 *03:01																										
12,800 20,683 2262 *02:01 *03:02																										
3,454 5,408 nn NA NA																										
3,016 4,861 nn NA NA																										
17,204 22,296 nn NA NA																										
18,506 23,824 2883 *04:01 *03:01																										
17,211 21,945 3042 *05:03 *04:02																										
6,700 8,209 2064 *05:01 *04:02																										

SA = single antigen beads. AE= heterodimer on cell line used for adsorption and elution. RC = recombinant cell lines. ae= HLA-DQ heterodimer on cell line used for adsorption/elution not same on SA bead (e.g. Adsorption/ Elution # 15, SA bead has DQA1*04:01/DQB1*04:02, cells line has DQA1*05:03/DQB1*04:02).

nn=not needed, NA=not applicable. Extra reactivity (MFI): E1 = 1000 - 2000, E2 = 2000 - 3000

Adsorption/Elution #59 (**Table 4**) shows the antibody targeting epitope 2010 that is defined by 45G + 46V. The amino acids for the recipient's two alleles DQB1*02:01, DQB1*03:19 at these positions are G + E and E + V. Given that we define the epitope by the combined amino acids, having the same amino acid at one position of the epitope and each of the recipient's alleles does not denote a self-epitope. This is clearly demonstrated by the negative beads 28, 29, 97, and 30 which have the recipient's DQB1*02:01 allele (**Table 2B**).

Each DQ epitope was defined by one or several combinations of two amino acids exclusively shared by either the alpha or the beta chains of SA beads' heterodimers positive with the antibody. In **Tables 3 and 4**, column position/amino acid, we list the least number of amino acids that are exclusive and may possibly define the epitope.

Fig. 2 shows a clear example of an eluted antibody targeting epitope #2001 found exclusively on the DQB1*02:01 and DQB1*02:02 chains and defined by one amino acid – leucine at position 52.

Figs. 3–5 illustrate three new epitopes (#2023, #2026, #2027) defined by 140A, 77R, and 77T respectively. It is noteworthy that one amino acid substitution at position 77 produces different antibodies, one against DQB1*02, 05 chains and the other against DQB1*03:01, 03:02, 03:03, 04, 06 chains.

MFI values for most Adsorption/Elution were moderate to strong, with 43% and 25% of MFI minimum values greater than 5000 and 10,000 respectively. And 89% and 73% of MFI maximum values were greater than 5000 and 10,000 respectively (**Tables 1 and 2**, MFI Min, MFI Max). For some Adsorption/Elution, we detected reactions against specificities that do not share the epitope. These extra reactions are denoted as E1 to E2 depending on the MFI level. Most reactions were marginally above 1000 MFI cutoff.

Three epitopes, one of which is newly defined in this study, were private epitopes exclusive to a single DQA or DQB chain, whereas the remaining 15 were public epitopes shared by 2–6 HLA DQA or DQB chains. Epitope #2017 and #2022, #2005 are examples of DQA and DQB private epitopes respectively (**Tables 1 and 2**). **Fig. 6** illustrates newly defined epitope #2022 found only on DQB1*05:01. Epitope #2018 is an example of a DQA public epitope (**Fig. 7**) and epitope #2010 is an example of a DQB public epitope (**Fig. 8**).

For some sera, up to three different antibodies were adsorbed then eluted. For example, three different antibodies were adsorbed from serum #10 (**Table 4**) and the eluted antibodies were determined to target epitopes #2023 shared by DQB1*02, 05, 06 (Adsorption/Elution 18), #2026 shared by DQB1*02, 05 (Adsorption/Elution 26), and #2004 shared by DQB1*05, 06 (Adsorption/Elution 41). In this case, serum #10, DQB1*05:02 is a mismatched antigen and therefore it is reasonable to deduce that all three antibodies were the result of immunological responses to this mismatched beta chain.

Some sera had reaction patterns that we were not able to define only by the epitope. For example, serum #17 (**Table 2**, Adsorption/Elution 19–23 and 70) showed a reaction pattern that cannot be completely explained by one shared epitope among all the positive heterodimers (DQB1*02, 04, 06, 03:01, 03:02, 03:03 and DQB1*02, 04, 03:01, 03:02, 03:03). Although all mentioned heterodimers share the aa 135D + [224Q] exclusively, bead #31 with the heterodimer DQA1*02:01/DQB1*02:02 is negative. Regardless, we assigned epitope #2024 and #2013.

Two different antibodies adsorbed from serum #33, one against DQA and another against DQB epitope, appear to have been an immunological response against the mismatched DQA1*02:01/DQB1*02:02 heterodimer (**Tables 3 and 4** Adsorption/Elution 7, 8, 31). The anti-DQA antibody targeted epitope #2017 (52H) located exclusively on the DQA1*02:01 and the anti-DQB antibody tar-

geted epitope #2001 (52L) located exclusively on the DQB1*02:01;02 chains (**Fig. 9**).

4. Discussion

The efficacy of using rHLA-DQ cell lines to adsorb specific antibodies is well illustrated in **Fig. 1**. Serum #4 has moderate to strong reacting antibodies with specificities to DQ, DR and DP. Using rHLA cell lines with the DQA1*02:01/DQB1*04:01 heterodimer for adsorption and elution produced an antibody reactive to beads with the DQB1*04, 05, 06 chains which share epitope #2007. DP, DR, and other DQ specificities were all negative.

We selected sera with DQ specificities at moderate to high MFI values from 30 patients who have experienced antibody-mediated rejection. The MFI values were indications that the adsorbed and eluted antibodies would be strong enough to retest with the single antigen beads. To our surprise, most eluted antibodies produced MFI values as strong as the initial un-adsorbed sera. This could be explained by a high concentration of the antibody in the serum, high concentration of the DQ antigens on the single antigen beads, or high affinity of the antibody to the target epitope. In our previous study of HLA class I epitopes, it was common that the eluted antibody reacted at approximately 25% the strength of the un-adsorbed serum [15].

Here, we define only six new epitopes and confirmed 12 of the 18 previously defined epitopes. This low number of epitopes defined could be due to the low number of sera we used, the origin of sera from one ethnic background, or that these are the most immunogenic epitopes. Furthermore, some epitopes that caused an immune response in the mouse, as seen in our previous study, may not trigger the same immune response in humans. The DQA1 and DQB1 epitopes identified here seem to be immunogenic and to elicit DQ antibodies in patients.

Unlike DR epitopes, where only the beta chain is polymorphic and therefore antibodies can be produced against epitopes on the beta chain of the DR heterodimer, DQ analysis is more complicated because antibodies are produced against the alpha or the beta chain.

Determining DQA specificities is shown for 8 out of the 30 sera tested. Antibodies appear to have been adsorbed by the DQA chains of the cell lines' heterodimers and the eluted antibodies were positive with heterodimers that have the same DQA chains (**Table 1**, Adsorption/Elution 1–16). All other DQA chains of the positive heterodimers share the same epitope. Every eluted antibody seems to be the result of an immunologic response to the DQA chains of the mismatched heterodimers (**Table 3**).

Similarly, determining DQB specificities is shown for 26 sera (**Table 2**, Adsorption/Elution 17–81). In almost all cases, the antibodies eluted from the cell lines seem to target DQB chains that share the same epitopes as that of the beta chain on the cell line.

In all eluted antibody tests, except one, Adsorption/Elution 53 the antibody reacted positive with the mismatched allele on which we defined an epitope by one or two amino acid substitutions at specific positions. However, the recipient's amino acids were different at the epitope positions (**Tables 3 and 4**). We defined epitopes by one or a combination of amino acid substitutions exposed on the HLA DQ allele. However, when in a combination, one amino acid that is the same in the recipient and donor at an epitope position does not denote that the epitope is a self-epitope. In **Table 2B** (Adsorption/Elution 60), the antibody adsorbed by cell # 2265 from serum #16 reacted positive with DQB1*04,05,06,03:02,03:03 that share epitope #2010 defined by 45G + 46V (**Table 4**). The recipient's alleles DQB1*02:01 and DQB1*03:19 have amino acids G + E and E + V at positions 45 + 46 respectively. Although the amino acid at position 45 is gly-

Table 2

(A) and (B). The results for DQB1 epitopes detected by eluted antibodies from the sera of 30 kidney transplant patients. The first column (Adsorption/Elution #) designate the assay # producing the results listed to the right followed by serum ID and the epitope #. The shaded squares indicate all heterodimers that were positive for each eluted antibody and the minimum and maximum MFI values are listed to the right (MFI Min, MFI Max). The cell ID and DQA1 and DQB1 chains of the rHLA DQ cell lines used are shown on the far right (#, DQA1*, DQB1*).

HLA DQB1 Epitopes											
HLA-DQ heterodimers on SA beads reactive with eluted antibody											
(Shaded squares show DQB1 chains sharing epitope)											
Lot 9 Bead	Antigen	DQB1	DQA1								
Adsorption/ Elution #	Serum	Epitope #									
17	31	2022 ^{new}									
18	10	2023 ^{new}									
19	17	2024 ^{new}	N								
20	17	2024 ^{new}	N								
21	17	2024 ^{new}	N								
22	17	2024 ^{new}	N	AE							
23	17	2024 ^{new}	N	ae	ae						
24	14	2025 ^{new}		E1	E1	E1					
25	14	2025 ^{new}					AE				
26	10	2026 ^{new}	AE								
27	35	2026 ^{new}				AE					
28	35	2026 ^{new}	AE								
29	29	2027 ^{new}			AE						
30	18	2001	AE								
31	33	2001	AE								
32	20	2003	E1								
33	20	2003					AE				
34	40	2003		AE	AE						
35	40	2003				AE					
36	40	2003					AE				
37	40	2003					AE				
38	40	2003						AE			
39	40	2003		AE							
40	40	2003				AE					
41	10	2004					AE				
42	15	2004					AE				
43	15	2004						AE			
44	15	2004							AE		
45	15	2004								AE	
46	24	2004									AE
47	13	2005									

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(continued on next page)

HLA DQ heterodimers on SA beads reactive with eluted antibody
(Shaded squares show DQB1 chains sharing epitope)

(B)		Antigen	DQB1	Lot 9 Bead		Adsorption/ Elution #	Serum	Epitope #	AE Cell Lines									
				DQA1	DQB1				AE	AE	AE	AE	AE	AE	AE	AE	AE	AE
48	9		2006	69	69	48	2006	2006										
49	26		2006	44	44	49	2006	2006										
50	26		2006	45	45	50	2006	2006										
51	26		2006	68	68	51	2006	2006										
52	27		2006	42	42	52	2006	2006										
53	1		2007	43	43	53	1	2007										
54	4		2007	41	41	54	4	2007										
55	23		2007	67	67	55	23	2007										
56	23		2007	66	66	56	23	2007										
57	39		2007	39	39	57	39	2007										
58	39		2007	40	40	58	39	2007										
59	16		2010	39	39	59	16	2010										
60	16		2010	38	38	60	16	2010										
61	32		2010	35	35	61	32	2010										
62	32		2010	36	36	62	32	2010										
63	32		2010	37	37	63	32	2010										
64	32		2010	38	38	64	32	2010										
65	7		2013	32	32	65	7	2013										
66	7		2013	33	33	66	7	2013										
67	7		2013	34	34	67	7	2013										
68	7		2013	35	35	68	7	2013										
69	7		2013	36	36	69	7	2013										
70	17		2013	37	37	70	17	2013	N	AE	E1	E2	E1	AE	AE	AE	AE	AE
71	37		2014	38	38	71	37	2014										
72	37		2014	39	39	72	37	2014										
73	37		2014	40	40	73	37	2014										
74	37		2014	41	41	74	37	2014										
75	37		2014	42	42	75	37	2014										
76	37		2014	43	43	76	37	2014										
77	38		2014	44	44	77	38	2014	E2	E2	AE							
78	38		2014	45	45	78	38	2014	E2	E2	AE							
79	40		2014	46	46	79	40	2014	E1	E1	AE							
80	30		2015	47	47	80	30	2015										
81	30		2015	48	48	81	30	2015										

SA = single antigen beads. New = new epitope beads.

ae= HLA-DQ heterodimer on cell line used for adsorption/elution not the same on SA bead (e.g. Adsorption/Elation # 79, SA beads 96 & 34 have DQA1*02:01/DQB1*04:02 &

DQA1*04:01/DQB1*04:02 respectively while the cell line has DQA1*06:01/DQB1*04:02). N = negative, m=not needed, NA=not applicable.

Extra reactivity (MFI): E1 = 1000 - 2000; E2 = 2000 - 3000.

Table 3

The DQA genotypes of recipients, donors and the mismatched DQA1 allele for each pair. The position/amino acid defining each epitope, is clearly different than the amino acid of each recipient's allele at the epitope position. This demonstrates that none of the epitopes listed is a self-epitope.

Adsorption/ Elution #	Serum	Epitope #	Position/ amino acid	DQA1 Chains sharing epitope	Recipient DQA1 alleles ^a	Recipient aa at epitope position ^b	Donor DQA1 alleles ^a	DQA1 mm ^c
1-3	18	2017	52H	*02:01	*01:02, *05:05:/09	S, R	*02:01, *05:05:/09	*02:01
4-6	22	2017	52H	*02:01	*01:01, *04:05, *01:02	S, S	*01:02, *02:01	*02:01
7-8	33	2017	52H	*02:01	*01:02, *05:05:/09	S, R	*01:02, *02:01	*02:01
9-10	35	2017	52H	*02:01	*01:02, *03:01:/02:/03	S, R	*01:02, *02:01	*02:01
11	2	2018	40G	*04,05,06	*01:01:/04:05, *01:02	S, S	*03:01, *05:01	*05:01
12	11	2018	40G	*04,05,06	*01:01:/04:05, *01:02	S, S	*05:01	*05:01
13-15	13	2018	40G	*04,05,06	*01:01:/04:05, *03:01:/02:/03	S, R	*01:01:/04:05, *05:05:/09	*05:05
16	21	2018	40G	*04,05,06	*02:01	H	*02:01, *06:01	*06:01

^a When exact allele is not known, two or more are listed separated by /.

^b Amino acids of the recipient's alleles at the position defining the epitope. Example: Adsorption/Elution #1, eluted antibody targets epitope 2017 defined by 52H. The amino acids at position 52 for the recipient's alleles are serine (S) and arginine (R) respectively. Therefore epitope #2017 defined by 52H could not be a self-epitope.

^c Only relevant mm chains are listed due to limited space.

Table 4

The DQB1 genotypes of recipients, donors and the mismatched DQB1 allele for each pair. The position/amino acid defining each epitope, is clearly different than the amino acid of each recipient's allele at the epitope position. This demonstrates that none of the epitopes listed is a self-epitope.

Adsorption/ Elution #	Serum	Epitope#	Position/amino acid	DQB1 Chains sharing epitope	Recipient DQB1 alleles ^a	Recipient aa at epitope position ^b	Donor DQB1 alleles ^a	DQB1 mm ^c
<i>HLA DQB1 Epitopes</i>								
17	31	2022 ^{new}	125S+126Q	*05:01	*03:01,*06:02	A+Q,G+Q	*05:01,*06:02	*05:01
18	10	2023 ^{new}	140A	*02,05,06	*03:02	T	*03:02,*05:02	*05:02
19-23	17	2024 ^{new}	135D+[224Q] ^d	*02,04,06,03:01,03:02,03:03	*02:02:/06,*05:02	G+Q,D+R	*02:02,*03:19	*03:19
24-25	14	2025 ^{new}	(71T)	*06:03:01,03:02,03:03	*02:02:/06,*04:02	K,D	*03:01,*05:01	*03:01
26	10	2026 ^{new}	77R	*02,05	*03:02	T	*03:02,*05:02	*05:02
27-28	35	2026 ^{new}	77R	*02,05	*03:02,*06:04	T,T	*02:02/ :06,*06:03	*02:02
29	29	2027 ^{new}	77T	*04,06,03:01,03:02,03:03	*02:02,*05:02:/05	R,R	*02:02,*03:01/ :02	*03:01/ 02
30	18	2001	52L	*02	*03:01,*05:02	P,P	*02:02,*03:01	*02:02
31	33	2001	52L	*02	*03:01,*05:02	P,P	*2:02/ :06,*06:04:/34	*02:02
32-33	20	2003	52P	*04,05,06,03:01,03:02,03:03	*02:01,*02:02:/06	L,L	*05:03/ :08,*06:01	*05:03 ^e
34-40	40	2003	52P	*04,05,06,03:01,03:02,03:03	*02:01	L	*02:01,*03:01	*03:01
41	10	2004	84E	*05,06	*03:02	Q	*03:02,*05:02	*05:02
42-45	15	2004	84E	*05,06	*02:01,*03:19	Q,Q	*02:02,*06:03	*06:03
46	24	2004	84E	*05,06	*03:01,*03:02	Q,Q	*03:02,*05:01	*05:01
47	13	2005	45E	*03:01	*03:02,*05:03	G,G	*03:01,*05:03	*03:01
48	9	2006	55P	*03:01:03:02,03:03	*05:01,*05:03	R,R	*03:01,*03:02	*03:02
49-51	26	2006	55P	*03:01:03:02,03:03	*02:02,*05:01	L,R	*03:03	*03:03
52	27	2006	55P	*03:01:03:02,03:03	*02:01,*06:02	L,R	*02:01,*03:02	*03:02
53	1	2007	55R	*04,05,06	*02:01:/02:/06	L	*02:02,*03:03	*03:03
54	4	2007	55R	*04,05,06	*03:01,*03:02	P,P	*06:02,*06:09	*06:02
55-56	23	2007	55R	*04,05,06	*03:01,*03:02	P,P	*02:01,*05:01	*05:01
57-58	39	2007	55R	*04,05,06	*02:01,*03:02	L,P	*02:01,*06:02	*06:02
59-60	16	2010	45G+46V	*04,05,06,03:02,03:03	*02:01,*03:19	G+E,E+V	*02:02,*06:03	*06:03
61-64	32	2010	45G+46V	*04,05,06,03:02,03:03	*03:01,*02:02	E+V,G+E	*02:02,*06:03	*06:03
65-69	7	2013	84Q	*02,04,03:01,03:02,03:03	*06:02,*06:04	E	*02:02,*06:04	*02:02
70	17	2013	84Q	*02,04,03:01,03:02,03:03	*02:02:/06,*05:02	Q,E	*02:02,*03:19	*03:19 ^f
71-76	37	2014	182N	*04,03:01,03:02,03:03	*02:01,*06:04	S	*03:02	*03:02
77-78	38	2014	182N	*04,03:01,03:02,03:03	*02:01,*05:03	S,S	*03:03,*06:01	*03:03
79	40	2014	182N	*04,03:01,03:02,03:03	*02:01	S	*02:01,*03:01	*03:01
80-81	30	2015	125S	*05	*02:01,*06:09	A,G	*02:01,*05:01	*05:01

^a When exact allele is not known, two or more are listed separated by /.

^b Amino acids of the recipient's alleles at the position defining the epitope. Example: Adsorption/Elution #80-81, the amino acids for position 125 for the recipient's alleles are alanine (A) and glycine (G) respectively. Therefore epitope #2015 defined by 125S is not a self-epitope.

^c Only relevant mm chains are listed due to limited space.

^d All aa positions are exposed on the surface of the DQB1 chain. When aa positions are not available on the 3D structures and therefore not known whether exposed or cryptic, they are placed within square braces.

^e DQB1*05:03 not on the SA beads panel, however shares 52P with positive chains.

^f DQB1*03:19 is not in the SA beads panel, however shares 84Q with positive chains.

cine (G) in both the mismatched and the recipient's allele DQB1*02:01, glycine at position 45 does not constitute a self-epitope because the epitope is defined by the combination 45G + 46V. Table 2B (Adsorption/Elution #60-serum #16) demon-

strates this clearly in that DQB1*02:01 is negative (beads 28, 29, 97 and 30).

Two other examples that clearly demonstrate this are shown in Figs. 6 and 8. Fig. 6 shows the antibody reacting positive with

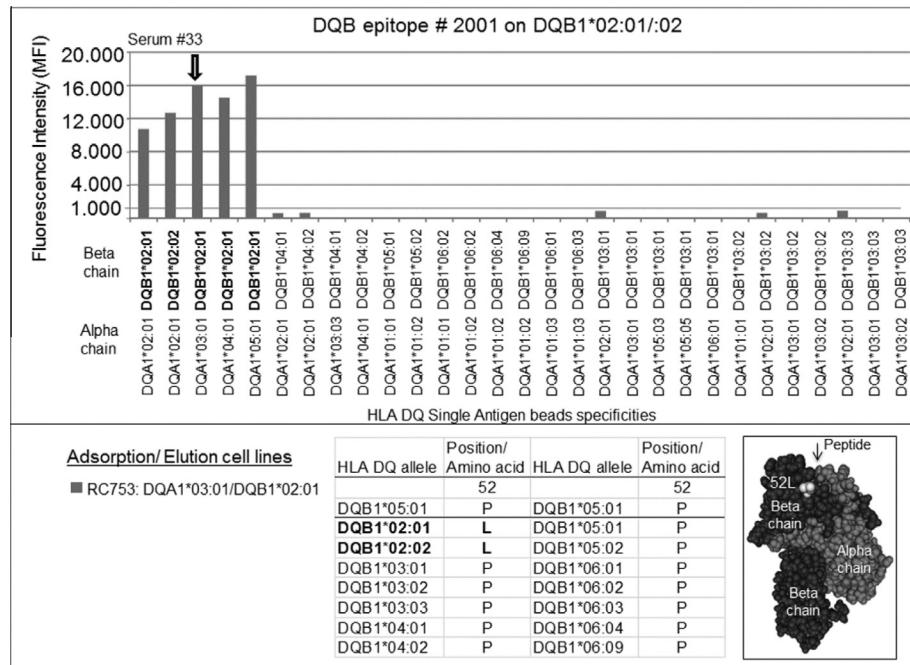


Fig. 2. Antibody from serum #33 adsorbed and eluted from rHLA SA cell line RC753, with the heterodimer DQA1*03:01/DQB1*02:01, was reactive only with DQB1*02:01/02 (Table 2A, Adsorption/Elution 31). This epitope, #2001, is defined by leucine (L) at position 52, shared exclusively by DQB1*02:01 and DQB1*02:02 (bolded). The arrow shows the bead with the same heterodimer as the rHLA cell line.

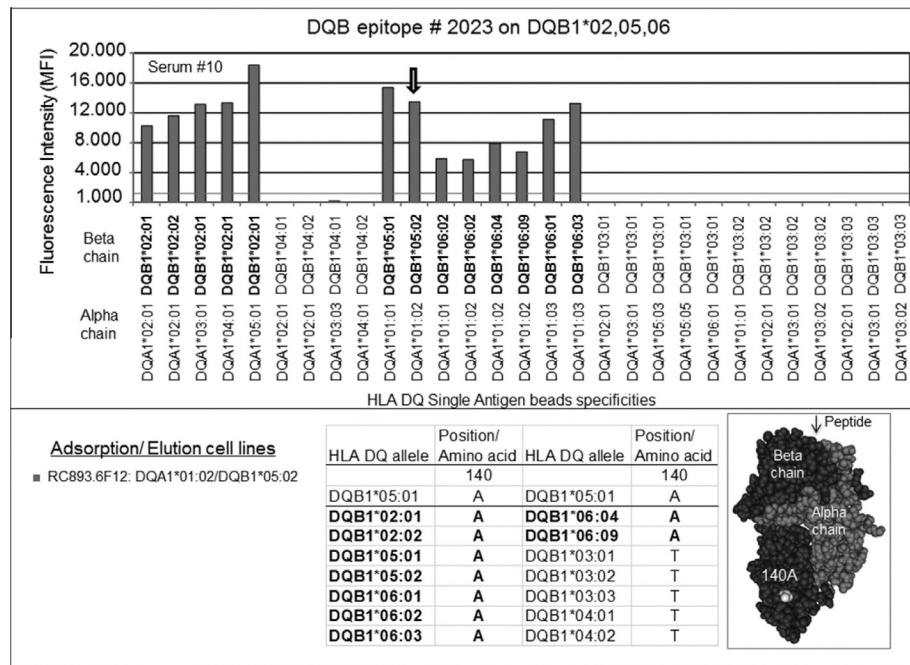


Fig. 3. Antibody adsorbed and eluted from serum #10 by the rHLA SA cell line RC893 (DQA1*01:02/DQB1*05:02) was reactive only with DQB1*02, 05, 06 (Table 2A, Adsorption/Elution 18). This epitope, #2023, is defined by alanine (A) at position 140, shared exclusively by alleles listed (bolded). The arrow shows the bead with the same heterodimer as the rHLA cell line.

DQB1*05:01 and all other beads are negative (Adsorption/Elution 17, **Table 4**). This antibody targets epitope 2022 define by 125S + 126Q. Although the recipient's alleles DQB1*03:01 and DQB1*06:02 have the same amino acid glutamine (Q) at position 126, it is clear that the heterodimers with the recipient's alleles are negative (**Table 2A**, beads 71, 38, 40, 39, 66, 67, and 41). Similarly, **Fig. 8** shows the recipient's allele DQB1*02:01 (Adsorption/

Elution #60, Table 4) is negative even though it has the same amino acid glycine (G) at epitope position 45.

Interestingly, we found two different antibodies adsorbed from the same serum, one against the alpha chain and the other against the beta chain and both seem the result of immunological responses to the mismatched DQA or the DQB chains. (Tables 1 and 2, Adsorption/Elution 7, 8 & 31). While this finding is not very

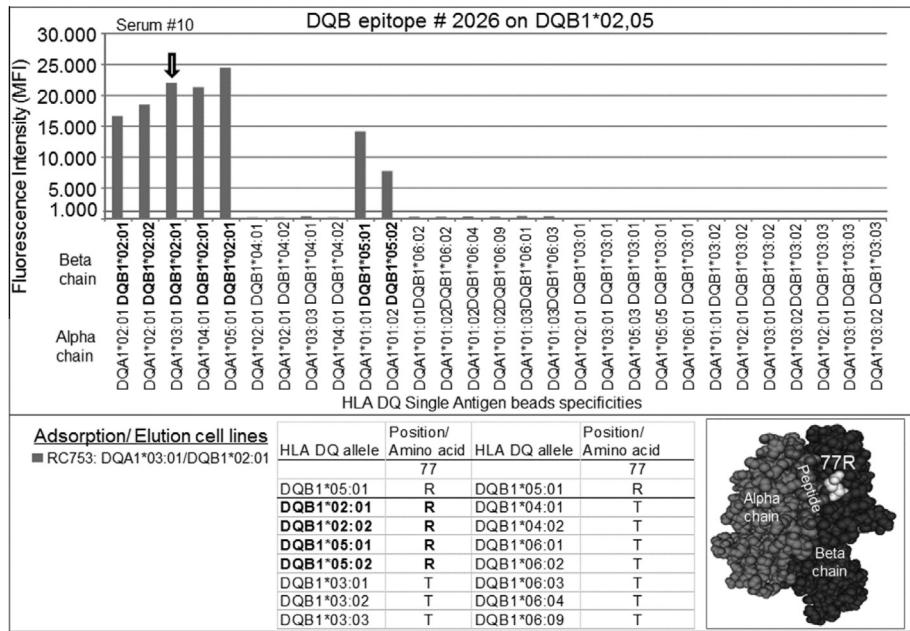


Fig. 4. Antibody from serum #10 adsorbed and eluted from rHLA SA cell line RC753, with the heterodimer DQA1*03:01/DQB1*02:01, was reactive only with DQB1*02,05 (Table 2A, Adsorption/Elution 26). This epitope, #2026, is defined by arginine (R) at position 77, shared exclusively by alleles listed (bolded). The arrow shows the bead with the same heterodimer as the rHLA cell line.

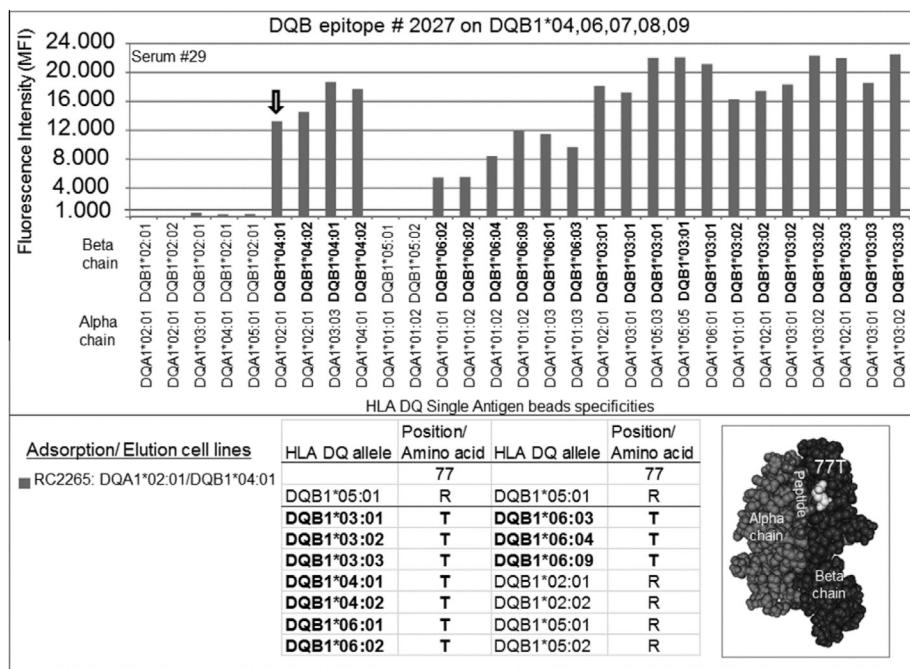


Fig. 5. Antibody from serum #29 adsorbed and eluted from rHLA SA cell line RC2265, with the heterodimer DQA1*02:01/DQB1*04:01, was reactive with DQB1*03:01,03:02,03:03,04,06 (Table 2A, Adsorption/Elution 29). Epitope #2027 is defined by threonine (T) at position 77, shared exclusively by alleles listed (bolded). The arrow shows the bead with the same heterodimer as the rHLA cell line.

surprising, it raises the question whether two antibodies, binding to the same heterodimer, will interfere with the binding of each other when in the same serum. Although these two antibodies, each targeting an epitope on opposite sides of the heterodimer may not interfere with each other in this case (Fig. 9), interference in binding may occur in other cases, where the epitopes are closer to each other cannot be ruled out.

The premise used here in defining the epitope is that an eluted antibody, presumed to be monoclonal and reacts positive with cer-

tain antigens on the single antigen beads must be targeting the same epitope on the alpha or beta chain. The epitope must be defined by one amino acid to three amino acids that are exclusive to the alpha or the beta chains on the positive beads. Epitopes are amino acids specially located at exposed positions and at distances from each other that fall within the binding span of the antibody [15]. The amino acids of the epitope form chemical bonds with amino acids in the Complementarity Determining Regions (CDR) loops in the paratope of the antibody.

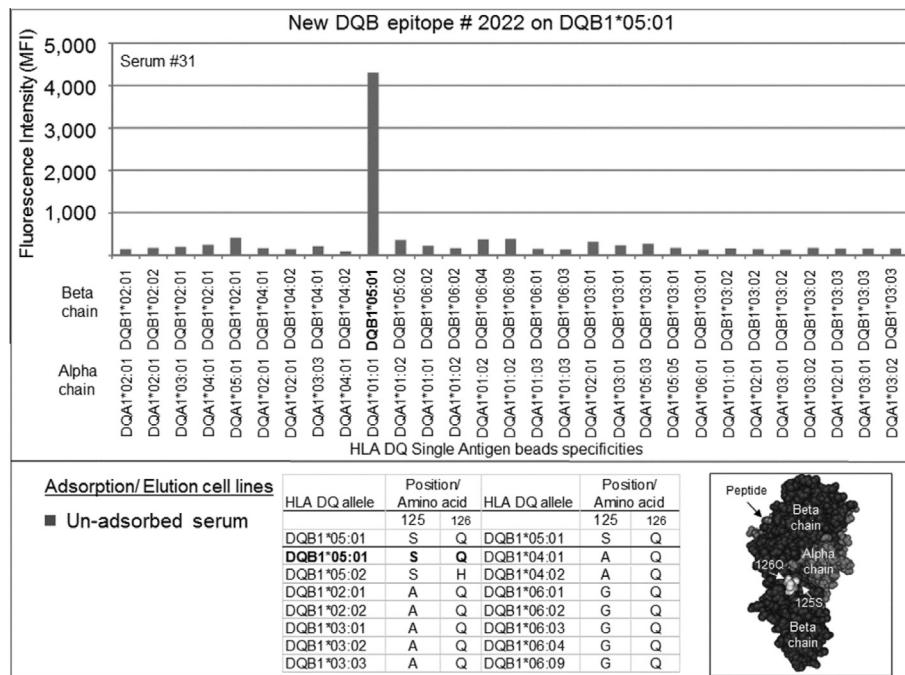


Fig. 6. Serum #31, tested un-adsorbed, has one specificity and presumed to have only one antibody reactive against the DQB1*05:01 chain (Table 2A, Adsorption/Elution 17). This epitope, #2022, is defined by a combination of serine (S) at position 125 and glutamine (Q) at position 126 on the DQ beta chain.

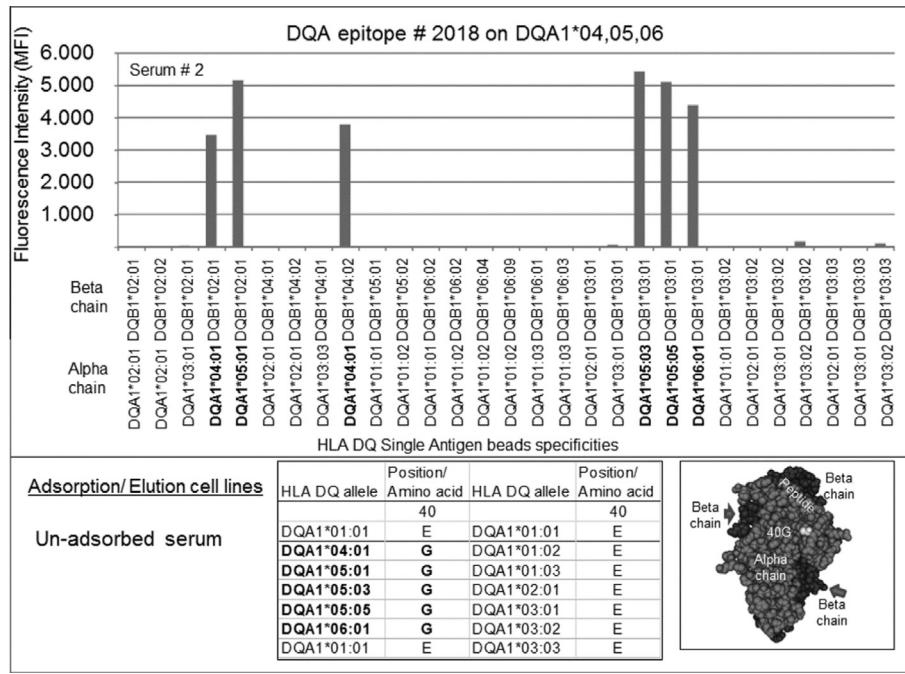


Fig. 7. Serum #2, un-adsorbed, presumed to contain one antibody specific for an epitope shared by alpha chains DQA1*04, 05, 06 (Table 1, Adsorption/Elution 11). This epitope, #2018, is defined by glycine (G) at position 40.

It is now accepted that the epitope, not the antigen, best defines the target of antibody and that the same epitope could be shared by 2 or more HLA antigens [1,15,16]. A donor specific antibody targeting a certain epitope on a mismatched antigen may also be reactive to many other antigens sharing the same epitope. The concept of mismatched epitopes or the differences in the number of aa mismatches as strong predictors of class II immunogenicity have been discussed by Duquesnoy et al. and Kosmoliaptis et al. [16,17]. If a

mismatched antigen is a contraindication to a transplant then a mismatched epitope should be a contraindication as well. Thus, defining the greatest number of DQ epitopes should be an important first step towards performing in-depth serum analysis using epitopes – epitope-based analysis has not reached widespread usage yet. Epitopes may become the aa structures to be considered when matching donors with recipients of organ transplants and are slowly getting incorporated in serum analysis software.

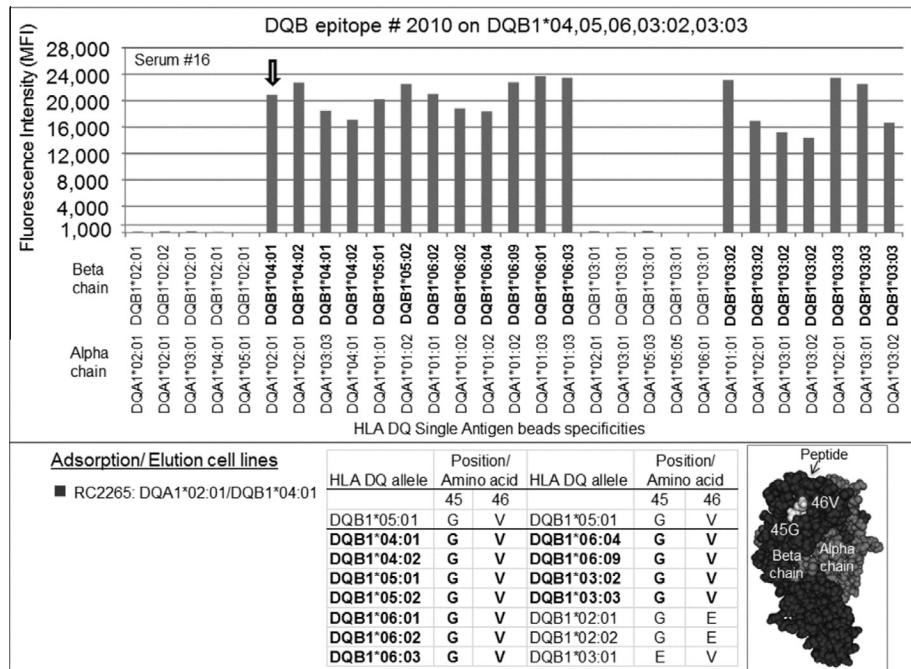


Fig. 8. Antibody from serum #16 that was adsorbed and eluted from rHLA SA cell line RC2265, which expresses heterodimer DQA1*02:01/DQB1*04:01 (Table 2B, Adsorption/Elution 60) shows strong reactions with all DQB1*04,05,06,03:02,03:03 chains (bolded). This epitope, #2010, is defined by the combination of glycine (G) at position 45 and valine (V) at position 46 on the beta chain. The arrow shows the bead with the same heterodimer as the rHLA cell line.

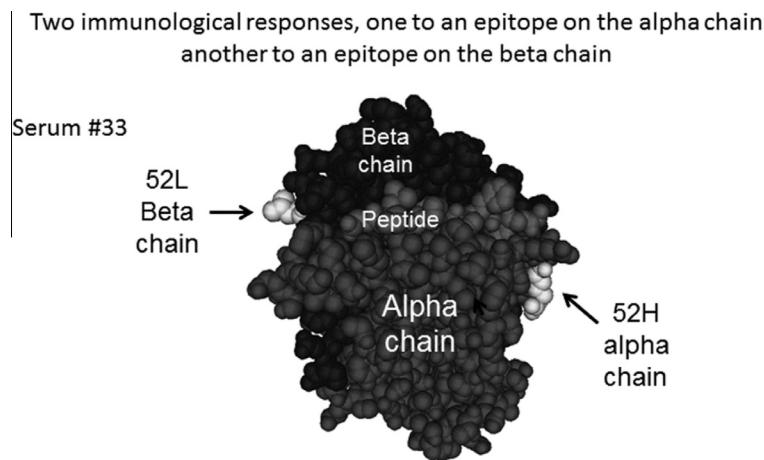


Fig. 9. Serum #33 contains two different antibodies, one against an epitope on DQA and another against an epitope on DQB chain. The anti-DQA antibody targeted epitope #2017 (52H) located exclusively on the DQA1*02:01 and the anti-DQB antibody targeted epitope #2001 (52L) located exclusively on the DQB1*02:01,:02 chains.

Some DQ epitopes have been previously defined. In our previous study, we used monoclonal antibodies and alloantibodies eluted from homozygous cell lines [1] to empirically define the DQ epitope. Duquesnoy has extensively defined epitopes based on the amino acid similarity among different DQ alpha and beta chains [16]. In this study, we sought to expand on our previous study by using rHLA DQ cell lines to adsorb and elute antibodies against DQ epitopes. A careful selection of appropriate cell lines, often two or more for each serum increases the chances of monoclonality of the eluted antibody.

The frequency and the strength of anti-DQ antibodies in the sera of solid organ transplant patients make the study of the DQ epitope more important. In a previous study from which we obtained our sera set, 44 patients with AMR and DSA and 20 losing their graft, anti-DQ antibodies were the most prevalent. Worthington et al. [8] noted the high frequency of DQ antibodies in patients who had rejected a transplant. DeVos et al. showed that 77% of pa-

tients in one study developed DQ DSA and stated that there is predominance of DQ DSA and increased evidence that DQ DSA have detrimental effect on the renal allograft outcomes [9]. Willicombe et al. showed the overall prevalence of DSA at 18.2% and 54.3% were *de novo* DQ DSA. The study highlights the importance of HLA DQ typing and matching in defining immunologic risk and that HLA DSA is associated with AMR, TG, and allograft loss. [7].

Knowledge of immunogenic HLA DQ epitopes should be useful in virtual crossmatching and may prove important with highly sensitized kidney patients as well as heart and lung transplants because of the insufficient time to perform actual crossmatches.

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