

Epitope Analysis of HLA-DQ Antigens: What Does the Antibody See?

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Background. Human leukocyte antigen (HLA)-DQ has emerged as the alloantibody most frequently associated with the generation of de novo donor-specific antibody (DSA), antibody-mediated-rejection, and unfavorable transplantation outcome.

Methods. The generation of HLA-DQ de novo DSA was interrogated in 40 transplant recipients who were immunologically naive before their failed transplantation. Eplet and epitope analyses were performed using HLAMatchmaker and Cn3D software.

Results. Ten DQA and thirteen DQB eplets or eplet combinations were identified. All but one revealed an epitope footprint that includes both the DQ α and DQ β chains. Four examples are illustrated in detail, representing a range of different epitope landscapes. A disparity between antigen density and mean fluorescence intensity values for some alleles within an eplet group was noted, with mean fluorescence intensity values of the lowest fluorescence bead being one tenth of the highest fluorescence bead, despite the fact that the amount of antigen on these beads were not significantly different.

Conclusion. Our data support the need for changing the manner in which HLA-DQ antigens and antibodies are evaluated for organ transplantation. The current nomenclature system does not reflect the true nature of HLA-DQ polymorphism. Moreover, epitope immunogenicity likely involves more than the mere presence of a specific eplet. Because our field contemplates the use of epitope matching as an approach to improve organ allocation and overall outcomes, it is imperative to have accurate characterization of the immunogenicity of each epitope. This will pave the way to identifying acceptable mismatches and will allow risk stratification for generating de novo HLA-DSA after transplantation.

Keywords: HLA-DQ, Epitope, HLA-antibody, de novo DSA.

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The interactions between human leukocyte antigens (HLA) and HLA-antibodies are complex. Contrary to the previously accepted concept, it is now clear that HLA antibodies recognize critical, polymorphic, short fragments of the HLA antigen rather than the complete antigen as one unit. These polymorphic subunits of the antigen can be unique to one HLA-antigen or be shared by several antigens that have different serologic specificities. Initial evidence for this phenomenon was observed in the early days of histocompatibility testing and was referred to as cross-reactive group (CREG) responses (1–4).

Several breakthroughs in understanding the HLA system allowed for better conceptualization of this phenomenon.

In the early 1990s, the ability to type for HLA-antigens by molecular techniques was acquired, demonstrating that HLA-antigens are in fact “families” of many *alleles*, with minor sequence differences, which belong to a particular antigen group as recognized by serologic typing. Around the same time, advances in crystallography techniques allowed to decipher the three-dimensional structures of HLA class I and class II molecules (5–7). It was then evident that many polymorphic amino acid residues concentrate in particular locations within the HLA molecule where they are exposed to antibody or T-cell receptor binding. Finally, in the early 2000s, testing for HLA antibodies using solid phase single antigen techniques improved the specificity and sensitivity of antibody identification, cumulatively providing us with

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new tools to appreciate the complexity and ambiguities of HLA antibody-antigen interactions. With the use of these tools, new evidence was brought forth, documenting that patients who were exposed to particular HLA mismatches had developed antibodies to a much wider range of antigens (alleles) compared with the original mismatched antigen expressed by their donors (8–11).

Two approaches were implemented in an attempt to identify the portion of the HLA molecule that is recognized by the antibody—the epitope. Duquesnoy et al. (12–16) in the early 2000 developed a theoretical algorithm, HLAMatchmaker, in which each polymorphic subunit of the HLA molecule was considered its own entity and termed originally triplets and later *eplets*. A more hands-on approach, using actual absorption or elution studies performed by the group of Terasaki et al. (17–19) was proven comparable with the theoretical algorithm. Importantly, both investigators refer to sequences of 1 to 3 amino acids as the critical difference between the antibody producer and the immunizer. Yet, the antibody recognizes a greater area with a footprint of approximately 15 Å radius (20), which constitutes the actual epitope. Consequently, analyzing antibodies against HLA class I antigens, Duquesnoy et al. reported that epitopes defined by eplet pairs always involved a non-self-eplet and a self-eplet that is shared between the immunizing antigen and the antibody producer (21–23). In fact, the data suggest an autoreactive component to the alloantibody response, which is in line with positive and negative selection processes taking place during B-cell development and receptor editing (24–26). The key concept of this theory is the need for a significant similarity between the immunizing HLA-antigen and the self-HLA-antigens expressed by the antibody producer, in the presence of a minor difference between the two.

We have explored the nature of structural epitopes for HLA-DQ molecules. To this end, we have studied de novo HLA-DQ antibodies generated in naive patients following failed kidney transplantation. Using high-resolution typing of both donor and recipient and results from single antigen class II Luminex assays, we were able to define the relevant eplets that caused the de novo antibody formation, to analyze the epitope footprint, to investigate the role of the nonself or self model, and to speculate about the use of this approach in determining donor compatibility for organ transplantation.

RESULTS

Forty recipients with de novo donor-specific HLA-DQ antibody (DSA) were analyzed as described in the *Materials and Methods* section. The complete list of epitopes identified in this study is presented in Table 1. These include 10 DQA eplets or eplet combinations and 13 DQB eplets (or combinations). Examples of four representative cases are given in detail in Figures 1 to 3. The first step of identifying the eplets is shown in Figure 1, where the information of the DQ-coated Luminex beads, the mean fluorescence intensity (MFI) values, the number of mismatched eplets, and their identity for DQ α or DQ β chains are provided. The eplet shared by all positive beads and the immunizer are highlighted by bold and italicized letters. The Cn3D software was used to highlight the exact location of the eplet

within an HLA-DQ molecule (Fig. 2 left). With the use of the “select by distance” command to identify a radius of 15 Å from the eplet, the complete epitope (antibody footprint) was highlighted in yellow as shown in Figure 2 center. This region, which is recognized by the antibody paratope, was then “detached” and reverted to the original color scheme to reveal the specific contribution of the DQ α and the DQ β chains (Fig. 2 right). Polymorphic amino acids composing the epitope were then highlighted and categorized as being accessible to antibody or TCR recognition (located in areas on the surface of the DQ molecule) or buried below the surface. This information is provided in Figure 3, together with the MFI values of the positive beads and along with the relative density of the antigen on each of these beads. The location of the polymorphic amino acids for each of the epitopes are illustrated in supplemental data (Figure 1, SDC, <http://links.lww.com/TP/B15>).

DQB77DR Eplet

Figure 1(A) presents the HLA-DQ antibody signature of a previously nonsensitized patient, demonstrating the formation of de novo HLA antibodies to seven DQ alleles (beads). The number of mismatched eplets (mm Ep) ranged between 3 and 8. All seven DQ alleles share one eplet, DQB77DR, that likely explains the positive reactivity seen against nonimmunizer HLA-DQ antigens. Other eplets are shared by some, but not all, positive beads and therefore are not likely to explain the antibody reactivity developed after exposure to the immunizer. Amino acid DQB77 is located approximately midmolecule, on the surface of the DQ β chain, close to the peptide-binding site (Fig. 2A left). Highlighting the antibody footprint—radius of 15 Å from amino acid DQB77 (Fig. 2A center) and then detaching this area and reverting to the original color code reveal that the epitope contains portions of both DQ α and DQ β chains as well as amino acids of peptide origin. To better understand the epitope-paratope interactions, we identified the polymorphic amino acids that are located on the surface on the molecule and therefore likely to come in contact with the antibody or the TCR (as defined by Cn3D), highlighted in bold and italicized fonts (Fig. 3A). The HLA-DQ specificities of the immunizer, the antibody producer, and positive beads are listed with their associated MFI value. The relative density of HLA-DQ molecules coating the beads is also provided, as the fluorescence emitted is dependent in part on the maximum amount of antigens attached to the bead. Interestingly, despite the fact that all positive beads share the DQB77DR epitope, the MFI values vary significantly, with the strongest bead showing an MFI value of 19,823 and the lowest positive bead having the value of 3,114. Yet, the density of DQ antigens on the lowest MFI value bead is 74% of that of the highest MFI value bead.

To test the nonself or self theory in this example, we compared the identity of the polymorphic amino acids among the antibody producer, the immunizer, and the DQ alleles coating the positive beads. Note that in this example, both the DQ α and DQ β chains contribute to the polymorphic or critical amino acid residues of the epitope. Indeed, for the most part, at least one of the immunizer alleles shares the same amino acids in the critical positions

TABLE 1. DQA and DQB eplets or eplet combinations as identified in this study

DQA	DQB
34HQ	34HQ.....41GR3
41GR3	14AM....26Y.....167HG 23L
47EK2	47EK2...56PRB.....5ILR 45GE5 45EV 45GV 52PL.....140T2 52PQ.....(84EV)
48LF	
56RR5	
56RB	
69T	
75SL4	75SL4....160AE 56PPA 56PPD 57LD.....70ED 70RT 77DR
129HA2	
160DD	
	84EV....87AF....90GI....125G....140A2

In some donor-recipient combinations, a clear identification of a single eplet was observed, whereas in other donor-recipient combinations, the same eplet was present with an additional eplet. It is not clear whether these represent different epitopes.

with the antibody producer; however, in position DQB75, both immunizer alleles have Valine, whereas the antigen producer has Leucine. All DQ alleles coating the positive beads also have Valine in this position. This observation raises the question regarding the relative significance of each polymorphic residue within the epitope; do they all contribute in the same way? Similarly, is there an absolute need for complete identity between the immunizer and antibody producer in critical positions?

DQB55-56PP Eplet

This patient shows positive responses to 12 different DQ-coated beads. The number of mismatched eplets ranges between 2 and 3. All positive beads express the 55 PPP and 56 PPD eplets (Fig. 1B). The eplet is located at the periphery of the DQB chain and, given a radius of 15 Å, includes amino acids from both DQα and DQB chains and the peptide (Fig. 2B). Similar to the previous case, a significant spread of MFI values for all positive beads is observed (range, 1,207–16,661). The allele that shows the lowest fluorescence has antigen density of 48% of the highest fluorescence allele (Fig. 3B). Remarkably, despite the fact that the antibody footprint covers both DQα and DQB chains, “critical” residues are located only on the DQB chain. In this example, consistent with the nonself or self theory, all polymorphic residues other than the core eplet are shared between the immunizer and the antibody producer.

DQA41GR Eplet

The third example is of an eplet located on the α chain of the DQ molecule. Seven positive beads show fairly low range of MFI values with one to two eplet mismatches between the antibody producer and immunizer and positive beads (Fig. 1C). The DQA56RR eplet is located in the periphery of the DQα chain, close to the peptide-binding groove. Highlighting the 15-Å radius area marking the epitope indicates that, similar to the other examples, all three structures, DQα and DQB chains as well as the peptide,

contribute to the antibody footprint (Fig. 2C). Interestingly, however, it is only the eplet itself DQA56RR that is located in the DQα chain where the rest of the epitope footprint resides mostly within the DQB chain. Of the five polymorphic residues, only three are identical between the antibody producer and immunizer, thus not fitting the nonself or self theory or at least questioning the role and function of all amino acids within the epitope.

DQA41GR Eplet

This is another example of an eplet located on the DQα chain showing six positive beads with two to five mismatched eplets (Fig. 1D). The shared eplet, DQA41GR, is located furthest from the peptide-binding groove and is the only case in this study in which the 15-Å radius area marking the epitope showed no involvement of the DQB chain (Fig. 2D). The density of DQ molecules on the lowest MFI bead is 71% of that of the highest MFI bead, with fluorescence range from 1,663 to 22,206 (Fig. 3). Of the five polymorphic amino acid residues within the epitope, four are shared consistent with the nonself or self theory.

DISCUSSION

HLA-DQ antibodies have emerged as the antibodies that are most frequently associated with the generation of de novo DSA, antibody-mediated rejection, and unfavorable transplantation outcome (27–30). Better understanding of the antigen-antibody interactions is required to minimize the risk of developing de novo HLA-DQ antibodies. In this study we analyzed DQ antibodies by interrogating de novo HLA-DQ DSA generated in immunologically naive transplant recipients, assessing their eplet and epitope specificities. Four examples of such analyses (A–D), representing a range of different epitope landscapes, are presented as follows: (A) The eplet is located on the DQB chain, yet the antibody footprint covers both DQα and DQB chains. (B) The eplet is located on the DQβ chain, and the antibody footprint covers both DQα and DQB chains; however, the “critical”

A DQB 77DR Eplet

DQA	DQB	MFI	mm Ep	DQB Eplets	DQA Eplets
DQA1*05:01	DQB1*02:01	19823	3	45GE5, 56LPA, 77DR
DQA1*01:01	DQB1*05:01	15747	7	14GL, 30HYV, 70GA, 77DR , 87AY, 116I, 125SQ
DQA1*03:01	DQB1*02:01	14096	3	45GE5, 56LPA, 77DR
DQA1*04:01	DQB1*02:01	12997	4	45GE5, 56LPA, 77DR	69T
DQA1*02:01	DQB1*02:02	7375	4	45GE5, 56LPA, 77DR , 135G
DQA1*02:01	DQB1*02:01	5618	3	45GE5, 56LPA, 77DR
DQA1*01:02	DQB1*05:02	3114	8	14GL, 30HYV, 57PS, 70GA, 77DR , 87AY, 116I, 125SH
DQA1*02:01	DQB1*04:01	Neg	0	Antibody Producer:
DQA1*02:01	DQB1*03:02	Neg	0	DRB1*11:01, DRB1*15:02
DQA1*04:01	DQB1*04:02	Neg	0	DQA1*05:01/DQB1*03:01
DQA1*01:02	DQB1*06:02	Neg	0	DQA1*01:03/DQB1*06:01
DQA1*02:01	DQB1*04:02	Neg	0
DQA1*01:01	DQB1*03:02	Neg	0
DQA1*02:01	DQB1*03:01	Neg	0
DQA1*03:01	DQB1*03:01	Neg	0
DQA1*01:02	DQB1*06:04	Neg	0	DRB1*07:01, DRB1*16:01
DQA1*01:01	DQB1*06:02	Neg	0	DQA1*02:01/DQB1*02:02
DQA1*02:01	DQB1*03:03	Neg	0	DQA1*01:02/DQB1*05:02
DQA1*03:01	DQB1*03:02	Neg	0
DQA1*03:01	DQB1*03:03	Neg	0
DQA1*03:03	DQB1*04:01	Neg	0
DQA1*01:02	DQB1*06:09	Neg	0
DQA1*01:03	DQB1*06:03	Neg	0
DQA1*05:03	DQB1*03:01	Neg	0
DQA1*01:03	DQB1*06:01	Neg	0
DQA1*03:02	DQB1*03:03	Neg	0
DQA1*06:01	DQB1*03:01	Neg	0
DQA1*05:05	DQB1*03:01	Neg	0
DQA1*03:02	DQB1*03:02	Neg	0

B DQB 55-56PP Eplet

DQA	DQB	MFI	mm Ep	DQB Ep	DQA Ep
DQA1*03:02	DQB1*03:02	16661	2, 55PPP, 56PPD ,
DQA1*05:03	DQB1*03:01	16135	3, 45EV, 55PPP, 56PPD ,
DQA1*03:02	DQB1*03:03	15512	2, 55PPP, 56PPD ,
DQA1*05:05	DQB1*03:01	15046	3, 45EV, 55PPP, 56PPD ,
DQA1*02:01	DQB1*03:03	14791	2, 55PPP, 56PPD ,
DQA1*06:01	DQB1*03:01	14349	3, 45EV, 55PPP, 56PPD ,
DQA1*02:01	DQB1*03:01	3235	3, 45EV, 55PPP, 56PPD ,
DQA1*03:01	DQB1*03:02	2619	2, 55PPP, 56PPD ,
DQA1*03:01	DQB1*03:01	2552	3, 45EV, 55PPP, 56PPD ,
DQA1*03:01	DQB1*03:03	2280	2, 55PPP, 56PPD ,
DQA1*02:01	DQB1*03:02	2170	2, 55PPP, 56PPD ,
DQA1*01:01	DQB1*03:02	1207	2, 55PPP, 56PPD ,
DQA1*02:01	DQB1*02:01	Neg	0	Antibody Producer:
DQA1*03:01	DQB1*02:01	Neg	0	DRB1*01:01, DRB1*13:02
DQA1*05:01	DQB1*02:01	Neg	0	DQA1*01:02/DQB1*06:04
DQA1*02:01	DQB1*02:02	Neg	0	DQA1*02:01/DQB1*02:02
DQA1*02:01	DQB1*04:01	Neg	0
DQA1*04:01	DQB1*04:02	Neg	0
DQA1*01:01	DQB1*05:01	Neg	0
DQA1*01:02	DQB1*05:02	Neg	0
DQA1*01:03	DQB1*06:01	Neg	0
DQA1*01:02	DQB1*06:02	Neg	0
DQA1*03:03	DQB1*04:01	Neg	0
DQA1*01:01	DQB1*06:02	Neg	0
DQA1*01:03	DQB1*06:03	Neg	0
DQA1*01:02	DQB1*06:04	Neg	0
DQA1*01:02	DQB1*06:09	Neg	0
DQA1*04:01	DQB1*02:01	Neg	0
DQA1*02:01	DQB1*04:02	Neg	0

FIGURE 1. A–D, Examples of four epitope analyses using the HLAMathmaker software. Each inset contains the antibody producer and immunizer HLA-DRB1, DQA1, and DQB1 high-resolution typing relevant for that particular case on the right side. The specificity of the HLA-DQ alleles used for antibody analysis (each line of DQA, DQB information) using solid phase single antigen Luminex assay is provided on the left end, next to the MFI values associated with each of those specificities. The bead information is sorted based on the MFI value from the highest to the lowest. The number of mismatch eplets (mm Ep) is in the following column followed by the description of these eplets for the DQ β and DQ α chains. The eplet shared by all positive beads and the immunizer is highlighted by bold and italicized letters and corresponds to the title of the specific inset. The four eplets presented are DQB 77DR, DQB 55-56PP, DQA 56RR, and DQA 41GR. HLA, human leukocyte antigen; MFI, mean fluorescence intensity.

C DQA 56RR Eplet

DQA	DQB	MFI	#Ep	DQB Ep	DQA Ep
DQA1*0302	DQB1*0303	6480	2	,56RR5,.....,160DD,,
DQA1*0302	DQB1*0302	6187	2	,56RR5,.....,160DD,,
DQA1*0301	DQB1*0201	4987	1	,56RR5,.....,
DQA1*0303	DQB1*0401	4803	2	,56RR5,.....,160DD,,
DQA1*0301	DQB1*0301	2738	1	,56RR5,.....,
DQA1*0301	DQB1*0302	2488	1	,56RR5,.....,
DQA1*0301	DQB1*0303	2075	1	,56RR5,.....,
DQA1*0101	DQB1*0302	Neg	0
DQA1*0503	DQB1*0301	Neg	0
DQA1*0505	DQB1*0301	Neg	0
DQA1*0501	DQB1*0201	Neg	0
DQA1*0601	DQB1*0301	Neg	0
DQA1*0401	DQB1*0402	Neg	0
DQA1*0201	DQB1*0301	Neg	0
DQA1*0401	DQB1*0201	Neg	0
DQA1*0201	DQB1*0401	Neg	0
DQA1*0201	DQB1*0402	Neg	0
DQA1*0201	DQB1*0302	Neg	0
DQA1*0102	DQB1*0602	Neg	0
DQA1*0101	DQB1*0602	Neg	0
DQA1*0102	DQB1*0502	Neg	0
DQA1*0102	DQB1*0604	Neg	0
DQA1*0103	DQB1*0601	Neg	0
DQA1*0201	DQB1*0202	Neg	0
DQA1*0201	DQB1*0303	Neg	0
DQA1*0102	DQB1*0609	Neg	0
DQA1*0101	DQB1*0501	Neg	0
DQA1*0201	DQB1*0201	Neg	0
DQA1*0103	DQB1*0603	Neg	0

Antibody Producer:

DRB1*01:01, DRB1*07:01
DQA1*01:01/DQB1*05:01
DQA1*02:01/DQB1*02:02

Immunizer:

DRB1*04:02
DQA1*03:02/DQB1*03:02

D DQA 41GR Eplet

DQA	DQB	MFI	mm	Eps	DQB Eplets	DQA Eplets
DQA1*05:01	DQB1*02:01	22206	5	45GE5, 56LPA	41GR3, 75SL4, 160AE	
DQA1*05:05	DQB1*03:01	21159	3	41GR3, 75SL4, 160AE	
DQA1*05:03	DQB1*03:01	20914	3	41GR3, 75SL4, 160AE	
DQA1*04:01	DQB1*02:01	10222	4	45GE5, 56LPA	41GR3, 69T	
DQA1*06:01	DQB1*03:01	6273	2	41GR3, 69T	
DQA1*04:01	DQB1*04:02	1663	2	41GR3, 69T	
DQA1*03:01	DQB1*02:01	Neg	0	
DQA1*02:01	DQB1*02:02	Neg	0	
DQA1*02:01	DQB1*02:01	Neg	0	
DQA1*02:01	DQB1*04:01	Neg	0	
DQA1*02:01	DQB1*03:03	Neg	0	
DQA1*02:01	DQB1*03:01	Neg	0	
DQA1*02:01	DQB1*04:02	Neg	0	
DQA1*02:01	DQB1*03:02	Neg	0	
DQA1*01:03	DQB1*06:03	Neg	0	
DQA1*01:03	DQB1*06:01	Neg	0	
DQA1*01:01	DQB1*05:01	Neg	0	
DQA1*01:02	DQB1*05:02	Neg	0	
DQA1*01:01	DQB1*06:02	Neg	0	
DQA1*01:02	DQB1*06:02	Neg	0	
DQA1*01:02	DQB1*06:04	Neg	0	
DQA1*01:02	DQB1*06:09	Neg	0	
DQA1*03:03	DQB1*04:01	Neg	0	
DQA1*03:01	DQB1*03:01	Neg	0	
DQA1*03:01	DQB1*03:02	Neg	0	
DQA1*01:01	DQB1*03:02	Neg	0	
DQA1*03:02	DQB1*03:02	Neg	0	
DQA1*03:02	DQB1*03:03	Neg	0	
DQA1*03:01	DQB1*03:03	Neg	0	

Antibody Producer:

DRB1*04:01
DQA1*03:02/DQB1*03:01

Immunizer:

DRB1*07:01, DRB1*11:01
DQA1*02:01/DQB1*02:02
DQA1*05:01/DQB1*03:01

FIGURE 1. (continued)

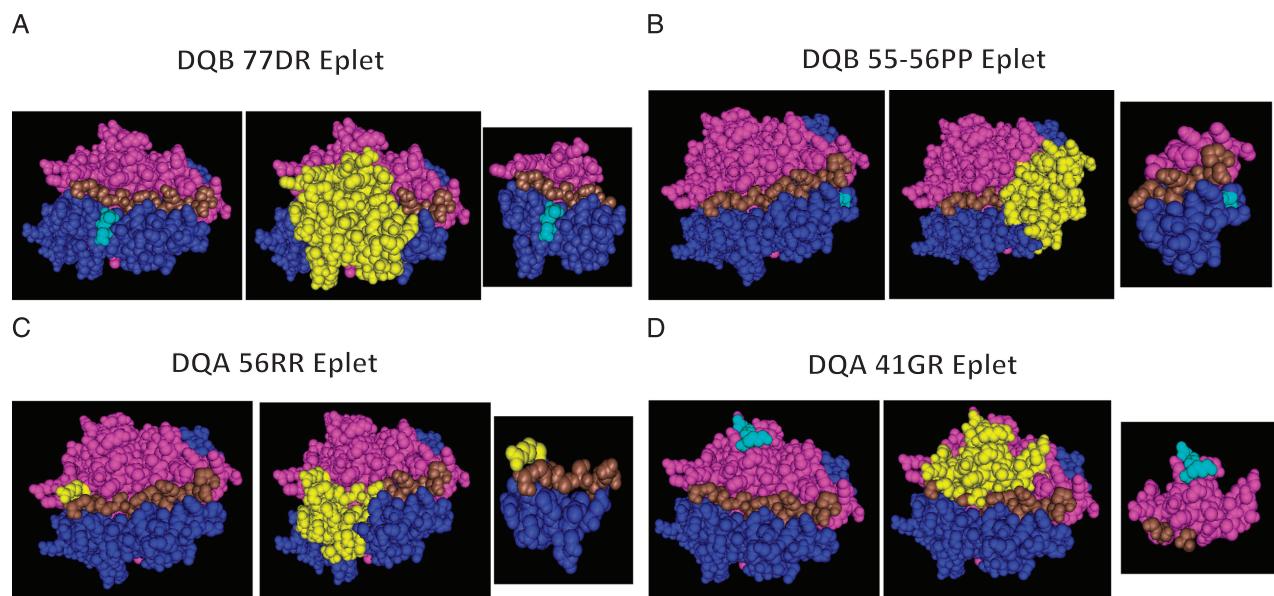


FIGURE 2. Four insets follow the same sequence as in Figure 1, each corresponding to one epitope identified. The left frame shows a three-dimensional structure of an HLA-DQ molecule in which the pink amino acids compose the DQ α chain, the blue amino acids compose the DQ β chain, and the brown amino acids represent the peptide lodged within the peptide-binding groove. The specific eplet identified is highlighted in light blue. The middle frame highlights the complete epitope by using the “select by distance” function of the 3nCd software to illustrate 15-Å radius from the eplet. The right frame isolates the eplet structure and presents it reverted into its original color scheme to identify the DQ molecule structures that are part of the functional epitope. HLA, human leukocyte antigen.

residues, considered to be more likely to contribute to antibody recognition, concentrate only in the DQ β chain. (C) The eplet is located on the DQ α chain with an antibody footprint covering both α and β chains. (D) A DQ α eplet has an antibody footprint covering areas solely on the DQ α chain.

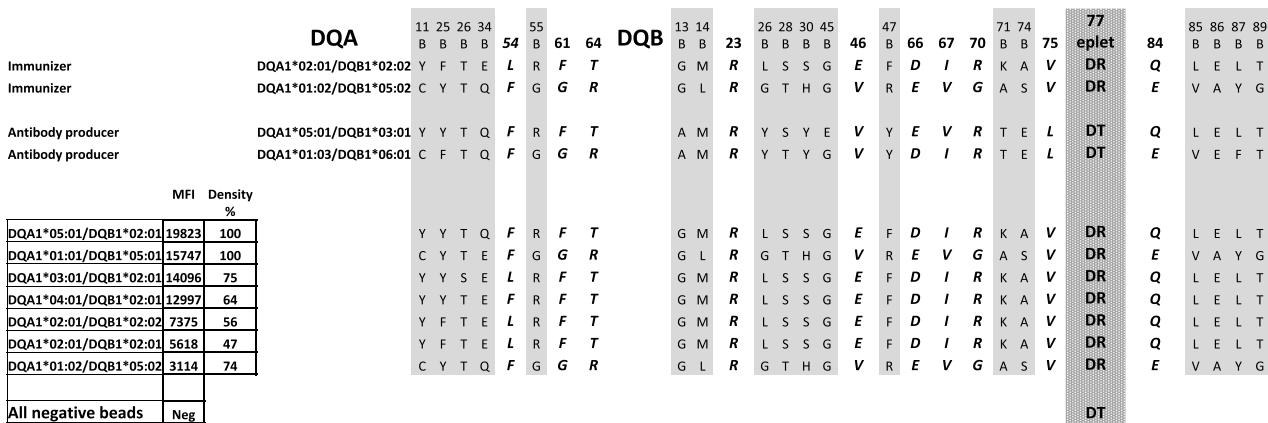
To the best of our knowledge, this is the first study attempting to identify not only the eplet associated with HLA-DQ antigen-antibody recognition but also the complete antibody footprint. It is clear from these results that the antibody does not make a distinction between the two chains of the DQ molecule and can recognize either or both in the same capacity. This is not a surprising observation because antibody analysis performed for class I molecules had shown similar data (23). In fact, in all five examples presented in that study, the surface residues within 15 Å of the eplet include amino acids of both the α 1 and α 2 domains of the class I molecule, corresponding to the α 1 and β 1 domains of a class II molecule. Yet, this is an important observation because current practices tend to look at HLA-DQ molecules as if they are composed of two independent components—DQ α and DQ β —and in fact, the only way to enter data in UNOS is by dividing the patient or donor typing or the antibody specificities into these two categories. The results presented in this study and supported by previous reports from our group (31–33) indicate that this practice is misleading and distorts the actual information regarding the specificity of HLA-DQ antibodies.

A puzzling observation was the disparity between the MFI values (strength) and the amount or density of antigen bound to the bead among alleles sharing the same eplet

(Fig. 3A–D). With the potential exception of example 3 in which all the MFI values were rather low, the MFI values of the lowest fluorescence bead was between 7% and 12% of the highest fluorescence bead, despite the fact that the amount of antigen on these beads ranged between 48% and 74%. For example, in Figure 3(A), two beads—DQA1*03:01/DQB1*03:01 and DQA1*01:02/DQB1*05:02—have the same percentage density of antigen (75% and 74%, respectively), both exhibiting the DQB77DR eplet; yet, the respective MFI values are 15,747 and 3,114. Artificial misalignment of HLA molecules on solid phase microspheres could potentially explain these observations. However, we think this hypothesis is not applicable in our case because analyzing different donor-recipient pairs, we observed scenarios in which a higher MFI ranking bead for one pair was among the lower ranking beads for another pair, despite the fact that the same eplet was implicated as the target of antibody binding. We therefore propose that although the eplet is critical for the definition of the immunogenic epitope, it is not sufficient to determine the “strength” of the response. Indeed, Kosmoliaptis et al. (34–36), in a series of elegant experiments analyzing surface electrostatic potentials, demonstrated that sequence information alone does not provide full insight into key determinant of epitope immunogenicity. In fact, even before sophisticated methodologies have been used, early serologic assays showed that HLA-specific alloantibodies could show heterogenic patterns of binding to the same amino acid sequence motifs, if those motifs were expressed on the background of different HLA-B alleles (37–39). Additional studies such as the use of soluble HLA molecules, assessing antibody affinity and avidity,

A

DQB 77DR Eplet



B

DQB 55-56PP Eplet

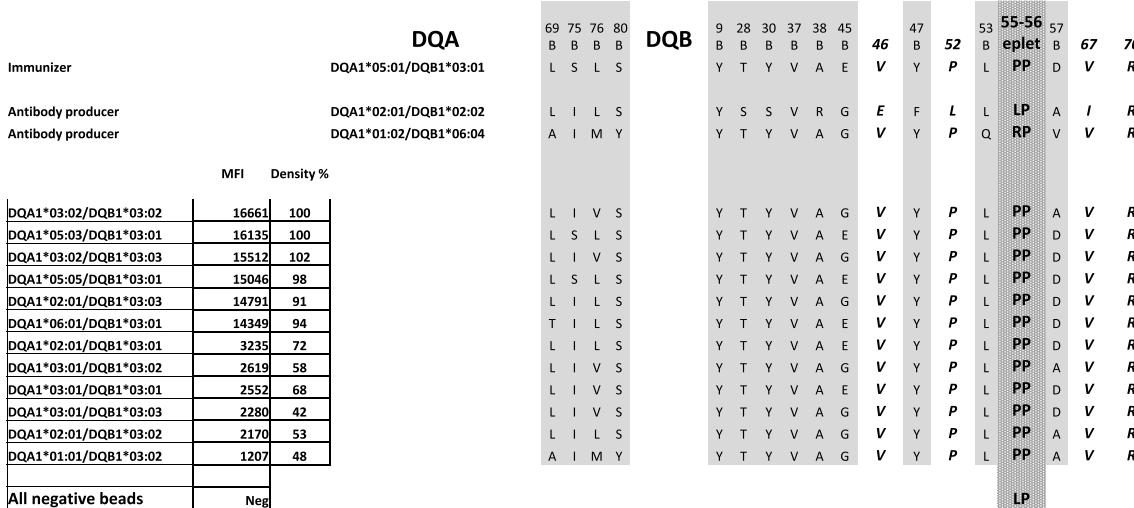


FIGURE 3. The epitope analysis interrogating amino acids that contribute to the epitope—15-Å radius from the eplet—as determined by the Cn3d software. Only positive beads and their polymorphic positions are described. Amino acids that are not exposed to the molecule's surface are shaded gray. Those that are exposed to the paratope are in bold and italics. HLA-DR and HLA-DQ typing of the immunizer and antibody producer for each case are listed at the top portion of each panel, to the right of the amino acid sequences. Below them is the allelic identification of the positive SA beads. MFI values, as presented in Figure 1, are listed to the right of the DQ alleles and adjacent to the % density of DQ molecule of each of these beads (assuming the highest MFI bead in each case represent 100% density). Given the significant difference between MFI values and % density as well as the known variability of the solid phase assays, those assays were repeated three times. No significant differences in the ratios were observed. Amino acids that are exposed to the surface, thus accessible to binding by an antibody or the TCR, are in bold and italics. Amino acids that are buried under the surface are indicated with the letter B below the AA number. HLA, human leukocyte antigen; MFI, mean fluorescence intensity.

proteomics, or site-directed mutagenesis are required to better understand this phenomenon.

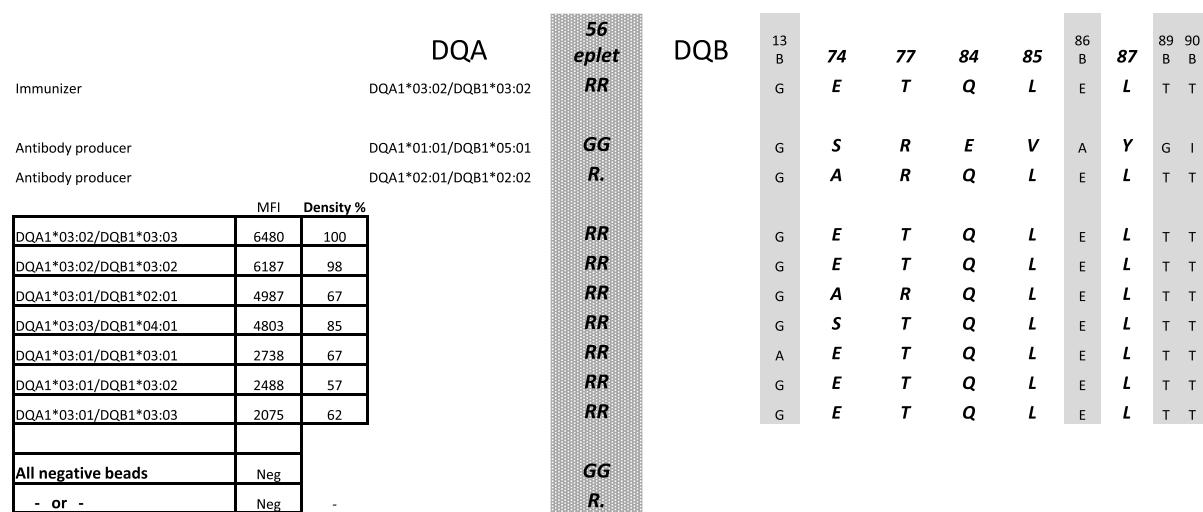
The non-self/self theory as presented by Duquesnoy et al. is very appealing and supported by compelling data from their studies (23, 40). Although our previous studies suggested a high frequency of non-self/self DQ chains involved in antibody development, our study of HLA-DQ epitope could not substantiate Duquesnoy's theory in its current form. Given the heterogeneity in binding observed in this study and data

presented by others, it is likely that additional parameters should be considered in refinement of the non-self/self theory to fully explain its applicability in clinical use.

In summary, we have presented additional evidence to support the need for changing the manner in which HLA-DQ antigens and antibodies are evaluated for organ transplantation. The current nomenclature system does not reflect the true nature of HLA-DQ polymorphism. Moreover, because our field contemplates the use of epitope matching as an

C

DQA 56RR Eplet



DQA 41GR Eplet

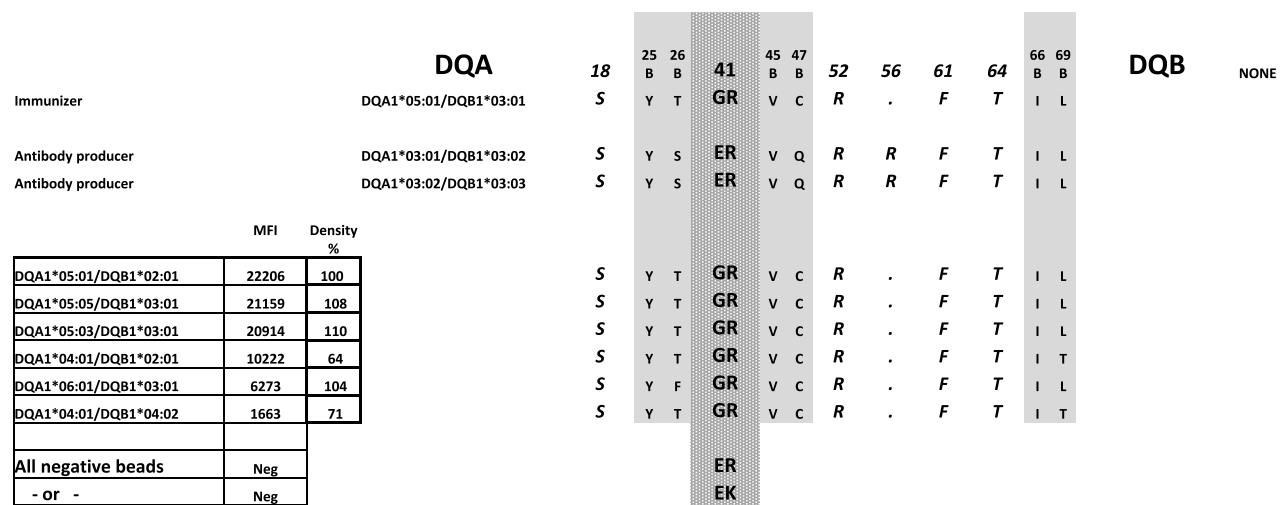


FIGURE 3. (continued)

approach to improve organ allocation and overall outcomes, it is imperative that we understand the true nature of an immunogenic epitope. This will pave the way not only to improve matching algorithms but also to predict the immunogenicity of HLA mismatched alleles so that acceptable mismatches can be identified and the risk of generating de novo HLA-DSA after transplantation can be better stratified.

MATERIALS AND METHODS

Forty kidney transplant recipients, transplanted and followed up at the Northwestern University Comprehensive Transplant Center, were evaluated for this study. Approval was obtained from the institutional review board. Patient inclusion criteria included a history of 0% panel reactive antibody (using solid phase-based assay) before their transplantation and kidney

transplant rejection leading to failure of the graft associated with the generation of de novo HLA-DQ DSAs. Availability of donor's and recipient's DNA for high-resolution typing was mandatory. Human leukocyte antigen typing was performed using HLA LabType SSO reagents for HLA-A, -B, -C, -DRB1, -DQA1, and -DQB1 (One Lambda, Canoga Park, CA). When required, ambiguity resolution was performed using micro-SSP high-resolution or allele-specific trays to ascertain the high-resolution typing of HLA-DRB1, DQA1, or DQB1. Assignment of common and well-documented alleles was based on Cano et al.(41).

Antibody testing was performed using LABScreenSingle Antigen(One Lambda) and LIFE CODES Single Antigen (Hologic Gen-Probe, San Diego, CA) HLA class II reagents following manufacturer's recommended protocol. Antibody specificity was documented as directed against DRB1, DQ α/β , or DP α/β molecules. Positive results were determined based on a flexible "cut-off" value between 1,000 and 2,000 MFI, based more on the architecture of the MFI histograms and the following eplet analysis. To allow direct comparison between MFI values on different beads, we evaluated the antigen density on

each of these beads using a monomorphic mouse anti-human antibody recognizing all DQ α/β combinations, clone B-K27 (ab47342, Abcam Cambridge, MA). Relative density was calculated in each case in relation to the highest MFI bead per patient, considered as 100%.

HLAMatchmaker software, a Microsoft Excel program that was developed to analyze antibody reactivity for eplets and eplet pairs, was downloaded from <http://www.HLAMatchmaker.net>. Patient information was entered as the “antibody producer,” and donor typing was entered as the “immunizer.” Patient’s antibody signature, including the MFI values, was populated into HLAMatchmaker such that eplets shared with the patient’s own antigens are automatically considered negative and are removed from consideration. The remaining eplets in the reactive alleles are presented such that eplets or eplet pairs that are shared with the immunizer can be identified. The Cn3d software (42) was used to visualize the three-dimensional structure of a crystallographic structure of an HLA-DQ molecule (<http://www.ncbi.nlm.nih.gov/Structure>); identify the exact location of the eplets, as determined by HLAMatchmaker; and calculate the footprint of the antibody CDR (15-Å radius from the eplet). (The notation system used in this article follows the one used by HLAMatchmaker. The author is advised that currently there is no single official notation system to describe HLA epitopes and that additional notations systems are used by Terasaki et al. or by the epitope registry Web site.)

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