The Complexity of Human Leukocyte Antigen (HLA)-DQ Antibodies and Its Effect on Virtual Crossmatching

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Background. We previously reported that in patients possessing human leukocyte antigen (HLA)-DQ-directed antibodies, the target molecule may include the patient's own DQβ chain if it is paired with non–self-DQα chain, thus forming a different DQ target. Herein, we sought to assess the breadth of this phenomenon.

Methods. Serum samples from 104 patients awaiting kidney transplantation, known to have DQ antibodies, were studied. Antibody identification was performed using luminex-based HLA class II single-antigen bead assays from two vendors; DQA1/DQB1 typing was performed using luminex polymerase chain reaction – sequence specific oligo prob hybridization (PCR-SSO) technology.

Results. A total of 71% of the 104 serum samples studied contained antibodies reactive against test beads coated with the patient's own DQα- or β-chain components. Of those, 35 patients (34%) exhibited antibodies to their own DQβ chain when in combination with non–self-DQα chains; and 64 patients (62%) had antibodies to their own DQα chain when in combination with non–self-DQβ chains. This is a striking observation.

Conclusions. To the best of our knowledge, this is the first systematic, high-resolution evaluation of DQ antibody repertoire. With the expansion of virtual crossmatching, particularly in the context of a national registry, the need for more detailed DQ antibody or antigen evaluation is critical to improve operational efficiency and patient outcomes.

Keywords: HLA antibodies, HLA-DQαβ, Virtual crossmatch.

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Human leukocyte antigen (HLA) class II antibodies were shown to have a detrimental role in transplant outcome (*1–8*). Until recently, however, the most commonly reported antibodies to HLA class II antigens were those targeting HLA-DR molecules. HLA class II antigens—HLA-DR, HLA-DQ, and HLA-DP—are expressed only on specific cell populations, unlike the HLA class I antigens that are expressed on all nucleated cells. Although both class I and II antigens share a similar three-dimensional structure, the polymorphic portion of the HLA class I molecule is encoded by one gene, whereas that of the HLA-class II molecule is a product of two separate glycoproteins (α and β), which are encoded by two separate genes (A and B, respectively) (*9*). Interestingly, the α chain for all class II antigens is less polymorphic than the β chain. In fact, the DRα chain is virtually not polymorphic having only two distinct proteins, whereas the DQα and DPα chains have moderate polymorphism compared with the relevant β chains (*10*). Consequently, common practice is to identify a class II molecule based on its β chain typing (DRB1, DQB1, and DPB1). However, although this practice is appropriate for DR molecules, it may be problematic for DQ and DP molecules, as herein discussed.

HLA-DR and HLA-DQ are known to be in strong linkage disequilibrium (*11*). That means that within a single ethnic or racial group, a certain HLA-DR molecule will usually be inherited together with the same HLA-DQ molecule. This phenomenon had created significant problems in identifying serologic HLA-DQ antibody specificities, because it was difficult to distinguish between antibody reactivity against HLA-DR antigens from that against HLA-DQ antigens. Consequently, until recently, the most commonly reported antibodies to HLA class II specificities were those directed at HLA-DR. It is only with the introduction of solid phase-based assays, and specifically, the single-antigen beads, specific reactivity against HLA-DQ was easily identifiable from that against HLA-DR (*12–14*). In fact, reactivity against DQ is common; in our center, more than 40% of the sensitized patients with reactivity against class II have antibodies against HLA-DQ specificities.

We have recently reported (*15*, *16*) several cases where antibodies to HLA-DQ molecules recognized not only the β

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chain portion of the molecule but also the α chain and that the epitope in question was most likely generated by a combination of the $\alpha\beta$ chains. We have also encountered many patients with what might have been viewed as expression of antibodies against their own HLA-DQ molecules. This, of course, is an impossible proposition, and indeed upon further investigation, we were able to demonstrate that although the antibodies recognized a molecule sharing the DQβ chain with the self-DQ molecule, the two DQ specificities differed in their DQα chains.

With this information at hand, we speculated that the routine testing approach that ignores the contribution of the DQα portion of the molecule can lead to scenarios in which patients' sera will be assigned as having antibodies against what may look like their own DQ specificity. Our concern was that in such cases, those so-called anti-self-antibodies be ignored because of high background or other technical issues. In reality, those will be "real" antibodies that may lead to unfavorable transplant outcome. Conversely, antibody specificities may be assigned where only a portion of this antigen group should be considered unacceptable, and other members of this antigen group should be immunologically acceptable for transplantation, thus precluding favorable outcome transplant from taking place.

RESULTS

Seventy-one percent (n=74) of the 104 serum samples studied contained antibodies reactive against test beads coated with the α- or β-chain components of patients' own DQ molecules. Specifically, 35 patients (34%) exhibited antibodies to their own DQβ chain when in combination with non–self-DQα chains; and 64 patients (62%) had antibodies to their own DQα chain when in combination with non–self-DQβ chains. Several examples are presented in Figures 1 to 4. Sera from 15 patients exhibiting moderate to strong antibodies against DQ molecules composed of non–self-DQα in combination with self-DQβ chain and non–self-DQβ in combination with self-DQα chain were used in flow cytometric crossmatch (FCXM) assays where target donor cells expressed the relevant DQ molecules. These patients were moderately sensitized, with class II panel reactive antibody ranging from 36% to 70%. Target cells were chosen, such that they did not express any class I or II antigens against which the recipient had antibodies other than those of interest. FCXM results demonstrated moderate to strong positive responses (channel shifts of 62 to 117 over the negative control using a 256-channel scale; data not shown).

Antibodies Against Self-DQα Chain When in Combination With Non–Self-DQβ

Figure 1 illustrates an antibody signature of a patient typed as DQA1*0101/DQB1*0501 and DQA1*0302/DQB1*0301 (serologic DQ5; DQ7, respectively). The bottom portion of the figure provides the median fluorescence intensity (MFI) values for 28 different HLA-DQ-coated single-antigen beads, with different combinations of DQA1 and DQB1 alleles (top portion of the figure). Interpretation of positive (+) or negative (−) reactions are presented at the center row of the upper frame. One bead (bead 47) coated with patient's self-DQA1 and DQB1 alleles combined is negative; there is no bead coated with the other patient's self-DQA1/DQB1 combination. MFI values clearly indicate antibodies against

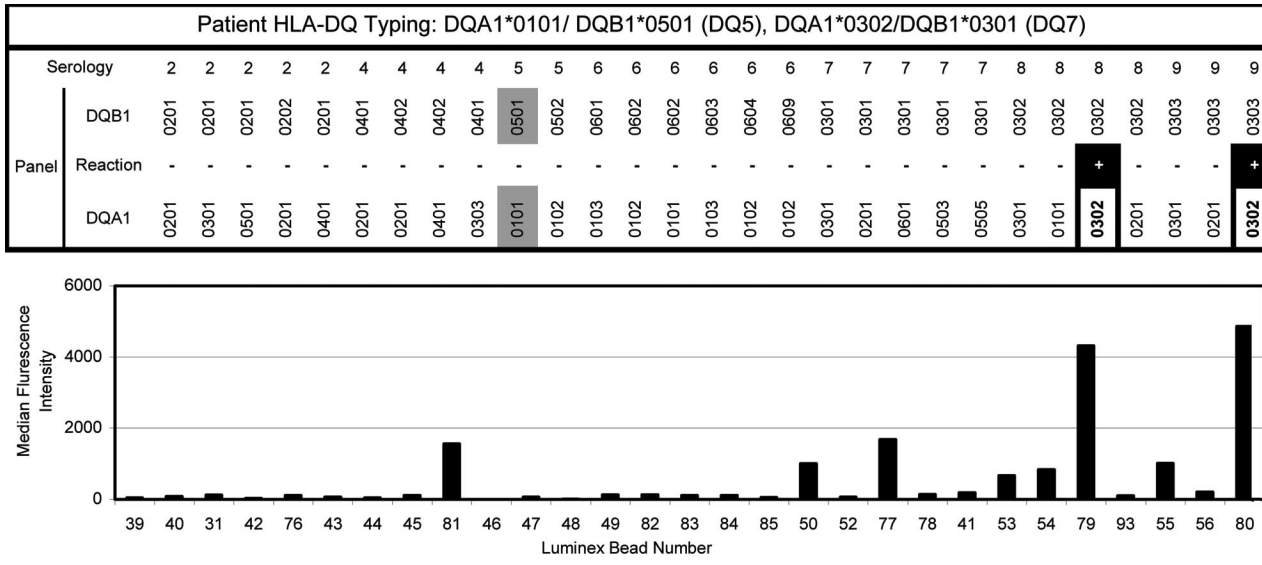


FIGURE 1. Antibodies against self-DQα chain when associated with non–self-DQβ. *Top* portion of the figure provides information about the specificities of the DQ single-antigen panel beads and their reactivity in this specific test. Panel specificities are provided above (DQB1) and below (DQA1) the actual reaction read outorinterpretation of positive or negative recognition. Serologic equivalents are provided at the *top line*. The beads containing the patient's own DQA1 and DQB1 alleles are boxed in *gray*. Positive results (+) are highlighted. The *bottom* portion represents a column chart of the median fluorescence intensity (MFI) values for each of the beads. A positive result was determined as MFI more than 2000. Beads carrying the patient's own DQA1-DQB1 allele combinations have low MFI values (there is no bead coated with the DQA1*0302/DQB1*0301 combination), whereas patient's own DQA1 allele that are in combination with a non–self-DQB1 allele show higher cutoff MFI values.

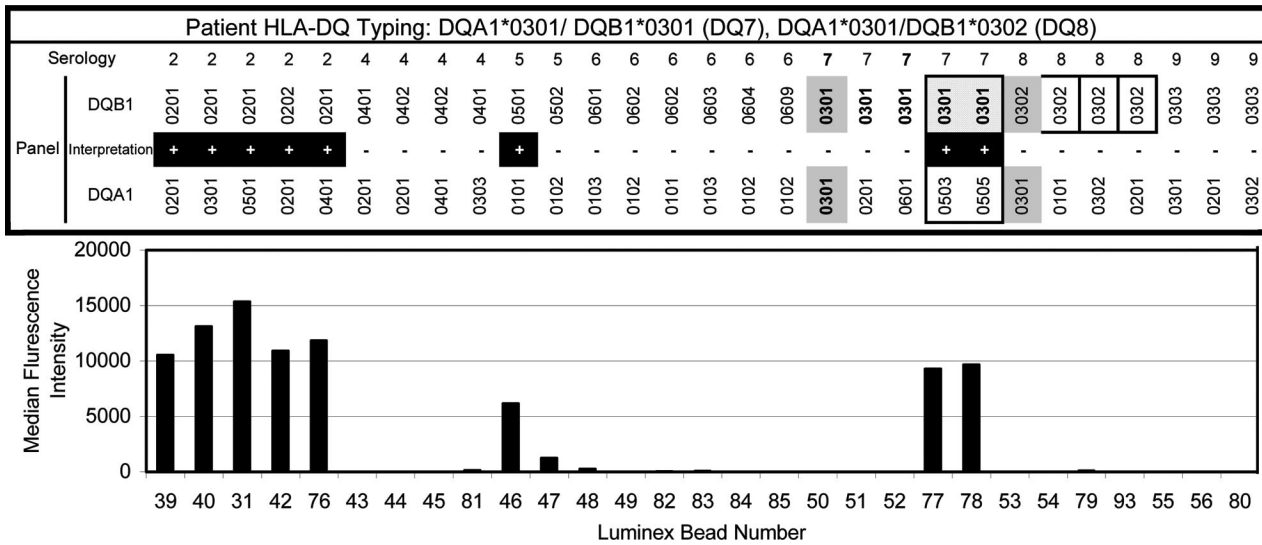


FIGURE 2. Antibodies against self-DQβ chain when associated with non–self-DQα. Data layout follows the one presented in Figure 1. Positive results (+) and the reactivity against self-DQβ associated with non–self-DQα (▨) are highlighted. As can be seen, beads carrying the patient's own DQA1-DQB1 allele combinations have low median fluorescence intensity (MFI) values. However, when patient's own DQB allele is in combination with a non-self DQA1 allele, the MFI values are higher then the established cutoff.

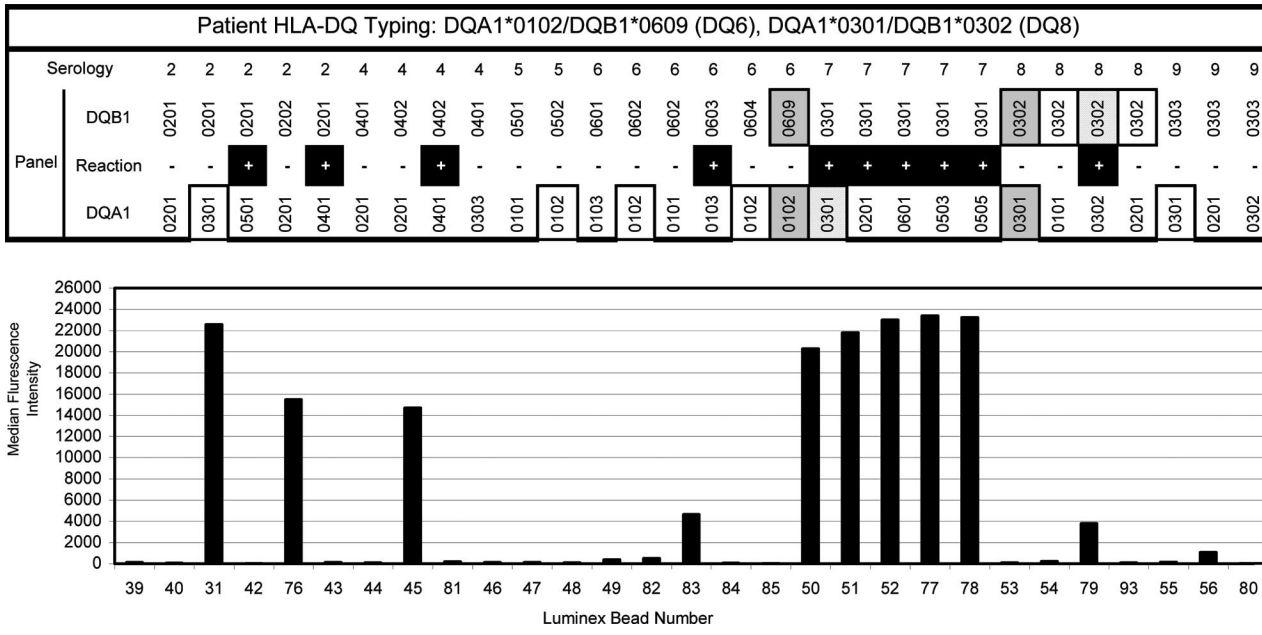


FIGURE 3. Antibodies against some of the patient's own DQβ and own DQα chains when in combination with non–self-α or -β chains. Data layout follows the one presented in Figure 1. Positive results (+) and the reactivity against self DQβ associated with non–self-DQα (▨) are highlighted.

HLA-DQ molecules containing one of the patient's own DQα chains (DQA1*0302), but only when in combination with non–self-DQβ chains—DQB1*0302 and DQB1*0303 alleles (beads 79 and 80, respectively).

Antibodies Against Self-DQβ Chain When in Combination With Non–Self-DQα

Patient typed as DQA1*0301/DQB1*0301 and DQA1*0301/DQB1*0302 (serologic DQ7; DQ8, respectively; Fig. 2). Data presented as indicated for Figure 1. Beads 50 and 53 representing patient's own DQ typing are grayed out. Patient had a clear positive response against all beads coated with DQB1*0201 regardless of the DQA1 associated with it and one positive bead coated with DQB1*0501 in combination with DQA1*0101. Two beads, 77 and 78, coated with the patient's own DQB1*0301, in association with non–self-DQA1, are positive and highlighted by dotted background.

Given the current panel of beads, it is not possible to unequivocally determine whether the reactivity is because of a sole response against the DQA1 alleles (DQA1*0503 or

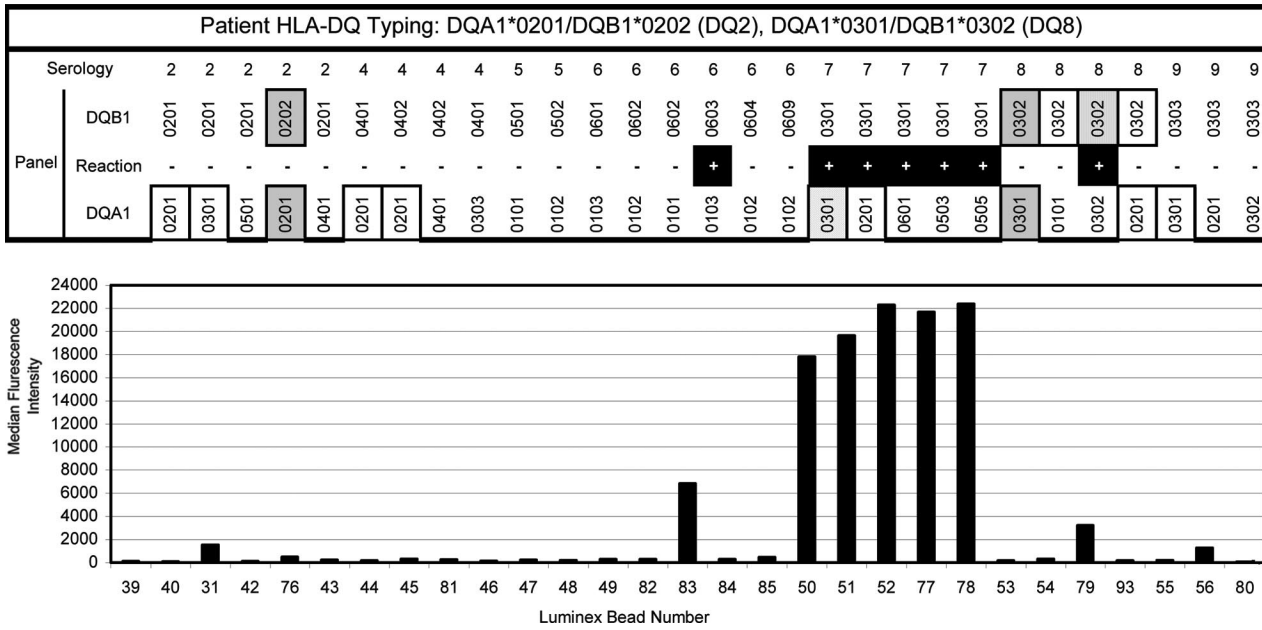


FIGURE 4. Antibodies against some of the patient's own DQβ and own DQα chains when in combination with non–self-α or -β chains. Data layout follows the one presented in Figure 1. Positive results (+) and the reactivity against self DQβ associated with nonself DQα (▨) are highlighted.

DQA1*0505) or whether the antibodies are directed at an epitope made of the combination of non–self-α with self-β chains. However, it is clear that the complete DQ molecule contains a β chain that is also present on the patient's own cells. The significant point remains that molecules carrying patients' own DQβ allele cannot be automatically considered negative for the purpose of assigning unacceptable antigens for United Network for Organ Sharing (UNOS) registries.

One quarter (25%) of the patients exhibited a combination of antibodies that react with beads coated with the patient's own DQβ or DQα chains in combination with non–self-α or -β chains. Figures 3 and 4 provide examples for such scenarios. The patient in Figure 3 was typed as DQA1*0102/DQB1*0609 and DQA1*0301/DQB1*0302 (serologic DQ6 and DQ8). MFI chart indicates positive responses to multiple beads including bead 50 that carries the patient's own DQA1*0301 but in combination with non–self-DQB1*0301 and bead 79 that carries the patient's own DQB1*0302 but in combination with non–self-DQA1*0302. This last reaction is interesting because it shows that the antibody recognizes an epitope that is formed by the combination of DQA1*0302 with DQB1*0302. Beads 53, 54, and 93 also express the DQB1*0302 but are negative; in fact, bead 53 expresses the same A/B combination as the patient himself. Bead 80 that expresses the DQA1*0302 but in combination with a different DQB1 is negative, indicating that the epitope is not confined to the DQα or the DQβ chains individually but recognizes an epitope formed by a combination of DQ$\alpha\beta$. The patient presented in Figure 4 has a different HLA type, DQA1*0201/DQB1*0202 and DQA1*0301/DQB1*0302 (serologic DQ2 and DQ8), and a different antibody signature. However, it also provides an example for antibodies recognizing antigens formed of part self-chains—bead 50—self-DQA1*0301 but with non–self-DQB1*0301; bead 51—self-DQA1*0201 but with non–self-DQB1*0301. Although this reactivity can be explained by antibodies to DQb1*0301, it again illustrates the possibility of self-DQα to pair with non–self-DQβ and be a target to antibodies. In addition, this case represents the same example of an epitope formed by DQA1*0302 with DQB1*0302.

TABLE 1. Frequency of antibodies against DQ molecules with self-components for each DQB1 serology equivalent groups and each DQA1 allele groups

Own DQB1 with non–self-DQA1			Own DQA1 with non–self-DQB1		
DQB1 serology group	N	DQB (%)	DQA1 allele group	N	DQA (%)
DQ2	12	32	A1*01	21	28
DQ4	1	3	A1*02	19	25
DQ6	4	11	A1*03	24	32
DQ7	17	46	A1*04	2	3
DQ8	1	3	A1*05	10	13
DQ9	2	5			

Table 1 summarizes the numbers and frequencies of patients with antibodies against HLA-DQ molecules composed of part self-components. According to our data, patients exhibiting HLA-DQ7 specificity have 46% chance of having antibodies against subspecificities of DQ7—composed of the same DQB1 allele but a different DQA1 allele. Table 2 provides the known or common linkage associations between DQB1 serologic equivalents and DQA1 alleles. Comparing these two tables, it is clear that the frequency of antibodies against self-DQB1 serologic equivalent (in combination with non–self-DQA1 allele) is directly related to the number of different potential DQA1 associations that can be formed. Thus, the highest frequency of antibodies against self-DQB1

TABLE 2. Common linkage associations between DQB1 serologic equivalents groups and DQA1 alleles

DQ serology	DQA1 allelic groups
DQ2	DQA1*02
	DQA1*03
	DQA1*05
DQ4	DQA1*03
	DQA1*04
DQ5	DQA1*01
DQ6	DQA1*01
DQ7	DQA1*03
	DQA1*04
	DQA1*05
	DQA1*06
DQ8	DQA1*03
DQ9	DQA1*02

with non–self-DQA1 was for HLA-DQ7 antigens; antigens share the DQB1*0301 allele but may partner with DQA1*03, *04, *05, or *06 alleles. Contrary to these results, the frequency of antibodies against the patient's own DQα chain in combination with a non–self-β chain is evenly distributed between the different DQA1 alleles groups with the exception of DQA1*04 that is mostly linked to DQB1*0402; and indeed, only two patients had such antibodies.

We further assessed putative antibody recognition sites or epitopes that serve as targets for the DQ antibodies analyzed in this study. Epitope assignment was based on combination of the Matchmaker algorithm (*33*) and manual analysis as described in the *Materials and Methods*. By using this approach, we were able to assign antibody recognition sites to all sera tested, identifying one or more such potential epitopes per sample. Overall we found that 90% of the samples had antibodies recognizing epitopes contributed by sequences of the DQβ chain. Thirteen percent of the samples had antibodies recognizing epitopes contributed by sequences of the DQα chain; and 33% of the samples had epitopes with sequences contributed both by the DQα and DQβ chains because they are aligned together in three dimensions. Examples for each of these three epitope groups are presented in Figure 5. Antibody reactivity patterns for three patients, P-I, P-II, and P-III, are presented at the top portion of the figure, and the putative epitopes are displayed at the bottom portion of the figure.

DISCUSSION

To the best of our knowledge, this is the first study were a systematic, high-resolution evaluation of DQ antibody repertoire—emphasizing the role of the different DQ chains—has been performed. By using this approach, we found that more than one third of the studied patients possess antibodies against DQ molecules composed of a DQβ chain identical to their own typing but in combination with a non–self-DQα chain. These antibodies caused moderate to strong donor-specific responses when used in FCXM assays against the relevant targets. This is a striking observation. Because the serologic nomenclature of HLA-class II molecules is dictated by the typing of the β chain, a self-DQβ with non–self-DQα will have the same serologic specificity as the self-DQβ/self-DQα. In such cases, the positive result obtained for antibody identification by the self-DQβ/non–self-DQα bead is usually dismissed as "high background/false positive," because "autologous-anti-HLA-antibodies" cannot exist. Moreover, the donor and recipient would have been considered serologically antigen matched, and transplantation could have been facilitated in the presence of an unrecognized donor-specific antibody.

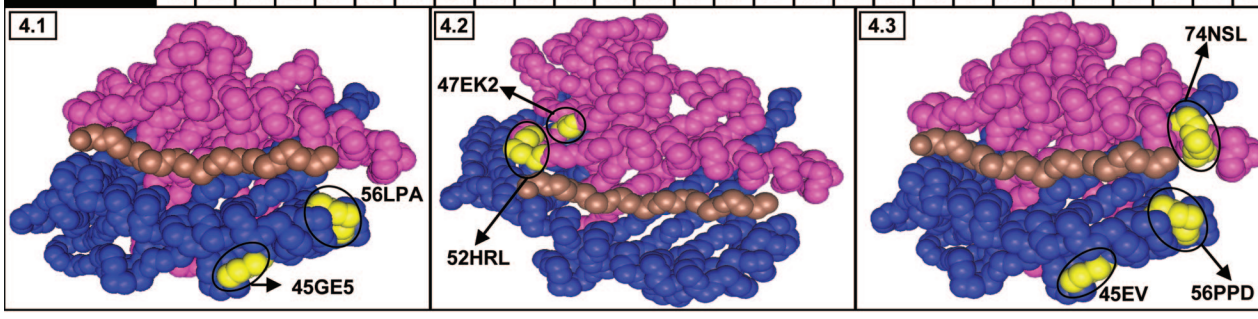
Our analysis identified a number of patients exhibiting antibodies to some, but not all, combinations of different DQα with the same DQβ chain. This was apparent mainly for patients with antibodies against HLA-DQ7 (DQB1*0301) and for few patients with antibodies against HLA-DQ2 (DQB1*0201 or *0202; Table 1). Indeed, these DQB1 alleles are known to pair with different DQA1 alleles to form complete DQ molecules, whereas other DQB1 molecules have a much limited range of DQA1 partners (*17*). However, we cannot exclude the possibility that our results are biased by the extent of reagents currently available. The ramification of such observation in the era of virtual crossmatching is that a patient may be considered to have donor-specific antibody to a particular donor and, therefore, be excluded from the "match-run" despite the fact that he has antibodies only to a subgroup of that DQ specificity (*16*, *18–20*). For example, a patient reported to have antibodies to DQ7 may in fact have antibodies only to a subgroup of this antigen family but will be excluded from final crossmatch against all donors typed as DQ7.

More than half of our patients expressed antibodies against DQ molecules composed of their own DQα chain in combination with a non–self-DQβ chain. Although this fact by its own is not likely to have a significant effect on the final antibody assignment or how we should evaluate the patient with regard to virtual crossmatch (XM), it raises the issue of how we consider DQ molecules. It is important to understand that although there are two separate genes that contribute to the DQ molecule (DQA1 and DQB1, respectively), the product of these two genes are entwined together to form a single molecule that can then be recognized by antibodies or the T-cell receptor. HLA class II molecules are similar in structure to HLA class I molecules (*21*, *22*). For HLA class I molecules, both extracellular domains of the protein, α1 and α2, have been shown to be targets for antibodies or T-cell receptor. These extracellular domains are the counterparts of the class II α1 and β1 outer domains, respectively. Yet, until now, common practice has been to consider only β1 typing results (because of the minimal polymorphic nature of the DRα chain). The data presented here clearly show that at least for DQ molecules (and likely for DP, in which the α chain is also polymorphic), both chains contribute to the complete structure of the molecule and should be considered for immunologic purposes.

Antibodies against HLA molecules, like all antibodies, recognize only a small portion of the complete molecule, referred to as epitope. Duquesnoy and Askar (*23*) and Duquesnoy and Marrari (*24*) have published an epitope-prediction software based on polymorphic triplicates within the HLA class I and II sequence motifs. Terasaki and coworkers (*25*, *26*) have defined epitopes by extensive elution studies. These studies demonstrated that both extracellular domains of the class I molecule—α1 and α2—individually or in com-

A

Serology	2	2	2	2	2	4	4	4	4	5	5	6	6	6	6	6	6	7	7	7	7	7	7	8	8	9	9	9
Bead N	39	40	31	42	76	43	44	45	81	46	47	48	49	82	83	84	85	50	51	52	77	78	41	54	93	55	56	80
DQB1	0201	0201	0201	0202	0201	0401	0402	0402	0401	0501	0502	0601	0602	0602	0603	0604	0609	0301	0301	0301	0301	0301	0301	0302	0302	0303	0303	0303
PI	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PII	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-
PIII	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
DQA1	0201	0301	0501	0201	0401	0201	0201	0401	0303	0101	0102	0103	0102	0101	0103	0102	0102	0301	0201	0601	0503	0505	0505	0101	0201	0301	0201	0302



B

Amino Acid Pos.	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
DQA1*010101	E	R	K	E	T	A	W	R	W	P	E	F	S	K	F	G	G	F	D	P	Q
DQA1*0103	-	K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DQA1*0106	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DQA1*0201	-	-	-	-	-	V	-	K	L	-	L	-	H	R	L	R	.	-	-	-	-
DQA1*030101	-	-	-	-	-	V	-	Q	L	-	L	-	R	R	-	R	R	-	-	-	-
DQA1*040101	G	-	-	-	-	V	-	C	L	-	V	L	R	Q	-	R	.	-	-	-	-
DQA1*0403N	G	-	-	-	-	V	-	C	L	-	V	L	R	X							
DQA1*0502	G	-	-	-	-	V	-	C	L	-	V	L	R	Q	-	R	.	-	-	R	-

FIGURE 5. Reactivity patterns for antibodies recognizing epitopes in the DQβ chain PI, DQα chain (PII) and epitopes composed of motifs in both chains (PIII). Epitope sites (*yellow*) on the surface of the α chain (*pink*) and β chain (*blue*) are represented on the Cn3D DQ model. PI typed as DQA1*0103/DQB1*0604; DQA1*0301/DQB1*0303 developed antibodies against HLA-DQ2. All beads carrying a DQB1*02 allele (whether it is the 0201 or the 0202 subtype; beads 39, 40, 31, 42, and 76) react with the patient's serum regardless of the DQA1 allele that associated with it to form the complete DQ molecule. This is a clear representation of an antibody recognizing an epitope specific to the DQβ chain alone. The two potential epitopes recognized by this patient's antibodies, as calculated manually and confirmed by Matchmaker, are highlighted in diagram 4.1 as 45GE5, 56LPA. PII typed as DQA1*0102/DQB1*0609; DQA1*0501/DQB1*0201 with antibodies against a variety of HLA-DQ serologic specificities, including some, but not all, DQ2 (beads 39 and 42), DQ4 (beads 43 and 44), DQ7 (bead 51), DQ8 (bead 93), and DQ9 (bead 56) antigens. However, it is clear that all the positive beads are coated with DQ molecules sharing the DQA1*0201 allele. Potential epitope includes 47EK2 and unique residues of DQA1*0201—positions 52 to 54 (diagram 4.2). PIII represents an antibody that recognize a specific combination of DQA1*0505/DQB1*0301, not reacting with DQA1*0505 or with DQB1*0301 when they are in combination with other alleles. The proposed epitope is, therefore, the combination of 74NSL on the α chain and 56 PPD on the β chain (diagram 4.3).

bination, can contribute to the immunogenic epitope. Similar data (*27–29*) have been reported for the class II extracellular counterparts, α1 and β1. Our finding that some of the DQ antibody recognition sites are directed specifically at DQα epitopes, some at DQβ epitopes, and some at epitopes formed by amino acid sequences from both chains are in line with these previously published studies. Moreover, a clear and elegant demonstration that epitopes can develop from unique interactions between the two-membrane distal domains of class II molecules was individually reported by Stroynowski et al. (*30*), Germain et al. (*31*), and Koeller et al. (*32*) by performing series of exon shuffling experiments.

The fact that 90% of the tested samples had antibodies recognizing epitopes contributed by sequences specific for the DQβ chain is reassuring and provides an explanation as to why, until today, it is the DQB1 typing that is considered the serologic equivalent. However, we do believe that the DQβ epitopes are affected by their partner α molecule and that it is the main contribution of the α chain toward antibody recognition. Indeed, only 13% of samples had antibodies that recognized epitopes contributed solely by sequences of the DQα chain. The low frequency of DQA1-specific epitopes may be the result of the known reduced polymorphism in the α chain compared with the β chain.

Regardless of the exact definition of the epitope recognized, the take home message of this article is that viewing DQ molecules at their current serologic definition is too simplistic and is not sufficient. Whether the self-component of the

DQ molecule (the α or the β chains) truly contributes to the antibody binding site or is merely in association with it—the fact remains that a more thorough analysis of DQ antibodies should be executed, such that our patients will not be disadvantaged. Currently, the UNOS database accepts patient and donor typing information only at the serologic level. Moreover, antibodies to DQ can be reported only as the serologic equivalent (DQβ chain only). Unacceptable and acceptable antigens are also reported at the same level of resolution. As we have demonstrated here, and by others (*29*), both polymorphic extracellular domains of the HLA-DQ molecule—α1 and β1—may contribute to the antibody recognition site. Thus, the current UNOS system does not allow entering information that may be significant for assigning compatibility of donor-recipient pairs at the appropriate resolution. Indeed, because of the strong linkage disequilibrium between the DR and DQ loci, if a high-resolution typing of the DR molecule is available, an HLA expert may correctly predict the DQα chain associated with a certain serologic DQ type. However, this is only a statistical assumption that may not always be accurate, and high-resolution typing of DR alleles is most always not available. Moreover, other personnel involved in accepting or declining a patient for the match-run are not usually educated in determining HLA high-resolution associations. Therefore, we believe that our observations may explain some of the apparent discrepancies seen by many centers between the virtual and actual crossmatch results. In this particular study, 40% of the patients had antibodies directed at DQ antigens. Of those, approximately one third had antibodies against "their own" DQB1 specificity. This means that approximately 15% of our patients may have been affected by the lack of attention to this phenomenon. Consequently, as we have demonstrated, the current system may significantly disadvantage up to 15% of the patients on the waiting list.

The currently available solid phase "single antigen" reagents for DQ antibody testing allow HLA laboratories to increase the resolution in which we assign specificities with no additional cost. On the basis of the results presented here, we encourage UNOS to require DQ antigen typing at a high or intermediate resolution for donor HLA-DQA/DQB genes instead of the current practice of reporting DQ serologic equivalents. In the mean time, it is advisable to consider the option of unique $\alpha\beta$ combination as B-cell XM results are analyzed. As we see the expansion of virtual crossmatching, particularly in the context of a national Kidney Paired Donation system, the need for more detailed antigen typing is critical to improve operational efficiency and patient outcomes. We further advocate the reporting of DQ-directed antibodies as specific pairs of DQα with DQβ chain combinations to account for the individual bead reactivity to facilitate accurate virtual XM assignment.

MATERIALS AND METHODS

Serum Samples

Serum samples from 104 patients awaiting kidney transplantation—mean age 49 years (range 24–71 years), mainly females (n=69; 66%)—were studied. Forty-eight patients had a history of a previous transplant, 42 had a history of pregnancies, and 9 had at least one transfusion event. The only inclusion criterion was the presence of known antibodies directed at HLA-DQ molecules.

HLA DQA1/DQB1 Typing and Single-Antigen Beads Assay

DQA1/DQB1 typing was performed using reverse Specific Sequence Oligo Probe hybridization method and detection by Luminex technology using the LABType SSO Class II DQA1/DQB1 Typing Test (One Lambda Inc., Canoga Park, CA). Data analysis was performed using the Visual software (One Lambda Inc.). Antibody identification was performed using LABScreen HLA class II single antigen beads kit LS2A01 (One Lambda Inc.) following manufacturer's protocol and then analyzed using HLA Visual software (One Lambda Inc.). Cutoff value for a positive response was set at 2000 MFI. Many of the antibody data were duplicated using a second manufacturer's reagents, Lifecodes LSA class II (GeneProb Inc., Stamford, CT).

Human Leukocyte Antigen Peptide Sequence and Epitope Assignment

Possible epitopes were postulated using the HLA-DQB1 and HLA-DQA1 peptide sequences obtained from the HLA informatics group of the Anthony Nolan website (*10*). We compared the results of our manual prediction of epitopes with candidate epitopes generated by the HLA-Matchmaker software (*33*). The three-dimensional models were constructed with the software Cn3D available at the NCBI website (*34*).

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