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Frequency of HLA-DP-specific antibodies and a possible new cross-reacting group

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ABSTRACT

Clinical studies have demonstrated that HLA-DP-specific antibodies can be detrimental to a transplanted kidney. The number of patients affected is proportional to the frequency of DP antibodies. We determined the frequency of HLA-DP-specific antibodies *en toto* and in the absence of cross-reactive DR antibodies. Of 650 waitlisted renal patients, 271 (42%) were reactive with HLA-DP antigens in solid-phase immunoassays. Of these 271 sera, 58 (21%) were negative for reactivity with cross-reactive DR antigens, and 16 (5.9%) had no class II antibody other than DP. Eliminating sera containing DR cross-reactive antibodies reduced the frequency but not the overall strength of DP antibodies. Although most DP antibodies were not expected to yield a positive cytotoxicity crossmatch, 2 DP-specific antibodies yielded cytotoxic crossmatch tests with titers of >512. The occurrence of HLA-DP-specific antibody differed significantly between previously transplanted (62%) and nontransplanted (38%) patients, but no difference was observed among patients categorized by race or sex. One serum demonstrated strong cross-reactivity between DP and DRB1*01:03 in the absence of DR1 or DR11 reactivity. Sequence alignments were performed and a possible new cross-reactivity between DRB1*01:03 and DP2, DP9, DP10, DP13, DP16, and DP17 was defined. Two additional sera confirmed this cross-reactivity.

1. Introduction

The timing and severity of rejection of a transplanted kidney often correlates with the amount of donor-specific antibody present in the patient's serum. Although human leukocyte antigen (HLA) class I (A, B, Cw) and some class II (DR, DQ) antigens have been known for decades to be the target of these antibodies, the clinical relevance of antibodies to antigens that have reduced expression is unclear. Among the least characterized of these is HLA-DP-specific antibody. HLA-DP antigens were first identified by cellular methods in 1980 [1], with DP-specific antibodies first reported in 1982 [2]. Although they were not originally thought to play a significant role in transplantation, several documented cases of rejection of A, B, C, DR, and DQ-matched transplants suggested a possible role of DP antigens in the rejection [3,4]. Some publications suggest that DP antigens are less immunogenic than other class II alleles [5]. However, DP has been demonstrated to be constitutively expressed on normal renal microvascular endothelial cells, implicating its ability to be a target of rejection [6]. This has led to further studies documenting transplant failure and rejection caused by DP antibodies [7–9].

Until recently, it was difficult for laboratories to identify DP-specific antibody. Not only did DR- and DQ-specific antibodies mask the reactivity of DP-specific antibodies, but also cross-reactivity between DP and DR complicated their identity. Sera that contain antibodies specific for DP, but not for DR or DQ, are uncommon. However, the development of solid-phase immunoassays with single antigen targets allowed for the detection and characterization of anti-DP antibodies [10]. Previous studies have demonstrated the presence of anti-HLA-DP antibodies in 5.1% of patients with functioning grafts [6] and 39% of patients with rejected grafts [11].

Analysis of DNA sequences has identified 2 epitopes shared between specific DRB1 and DPB1 antibodies [12], which explains the common co-occurrence of DR- and DP-specific antibodies. One epitope present on DR11 is shared with DP2, DP3, DP9, DP10, and DP14. A second epitope is shared between DR1 and DP1, DP4, DP5, and DP11. These epitopes were established through the equivalence of residue 56 in DPB1 with residue 58 in DRB1.

In this paper, we report the frequencies of DP-specific antibodies in 650 patients awaiting kidney transplantation and identify a possible new cross-reactivity observed between DRB1*01:03 and DP2, DP9, DP10, DP13, DP16, and DP17.

2. Subjects and methods

2.1. Study group

Antibody data were obtained for a unique serum from each of 650 patients awaiting kidney transplantation. The antibody assays

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Table 1
Demographics

DP-specific antibody	*N* (%)	Previous transplants	Race				Sex
		Yes *N* (%)	White *N* (%)	Black *N* (%)	Hispanic *N* (%)	Other *N* (%)	Male *N* (%)
Negative	379 (58)	150 (40)	241 (64)	91 (24)	18 (5)	29 (8)	180 (47)
Positive	271 (42)	167 (62)	169 (62)	72 (27)	10 (4)	20 (7)	128 (47)
Total	650	317	410	163	28	49	308
p		<0.0001	NS				NS

NS, not significant.

were performed as routine periodic monitoring of patients on the renal waitlist. The demographics of the study group are presented in Table 1.

2.2. Solid-phase immunoassays

Sera were tested using the LABScreen single antigen (SA) HLA class II antibody detection test (One Lambda, Canoga Park, CA) on a Luminex® 100 IS fluoroanalyzer (Austin, TX). Reactions with normalized values of ≥500 MFI (Mean Fluorescence Intensity) were considered positive. The DPA1/DPB1 antigens present in the SA kit are presented in Table 2. For each patient, the strongest reacting single antigen bead serum was used for analysis to maximize the opportunity of detecting DP-reactive sera.

2.3. Sequence alignments

HLA allele sequence comparisons were performed using the Sequence Alignment tool on the IMGT/HLA Database. Sequences of DRB1*01:01:01 and DRB1*11:01:01, which cross-react with certain DPB1 antibodies, were aligned with the sequence of DRB1*01:03, the proposed new cross-reactive antigen, and DPB1 alleles suggested to react with DRB1*01:03. Additionally, a DPB1 allele not thought to cross-react with DRB1*01:03 was aligned, namely DPB1*03:01, to illustrate differences from the other DPB1 alleles at the epitope being examined. The sequences for DRB1*04:02 and DRB1*1301 were later added to the alignment when they were observed to be reactive in a patient serum thought to display DRB1*01:03/DPB1 cross-reactivity.

2.4. Crossmatch tests

To demonstrate the clinical relevance of DP-specific antibody, B cell complement-dependent cytotoxicity (CDC) crossmatch tests were performed as previously described [13]. Briefly, purified B cells were tested in a one-wash CDC crossmatch where positive reactions were defined as reactions yielding a score of 2 (10–20% cell death) or greater. Four crossmatch tests were performed with patient sera exhibiting strong DP-specific antibody to assess the antibody strength. Target cells from healthy individuals were selected to avoid or minimize non-DP-specific reactivity. Antigens present in the panel phenotypes but not in the patients' phenotypes were identified and reactivity with beads bearing those antigens was noted (Table 3). It has been previously demonstrated that antibodies reacting on solid-phase immunoassays at less than 10,000 mean fluorescence intensity (MFI) are not sufficient to yield a positive CDC crossmatch [13]. Therefore, the DR13 and DQ6 mismatches present in the donor for crossmatch 2 were judged to be insufficient, collectively, to yield a positive CDC crossmatch, indicating that any reactivity seen on the test would primarily be caused by DP-specific antibodies.

Table 2
DPA/DPB allele combinations in single antigen kits

DPA1	DPB1
02:01	01:01
01:03	02:01
01:03	03:01
01:03	04:01
01:03	04:02
02:01	05:01
02:01	09:01
02:01	10:01
02:01	11:01
04:01	13:01
02:01	14:01
02:01	15:01[a]
02:01	17:01
02:01	18:01[b]
02:01	19:01
02:01	23:01[b]

[a]This combination was available in 244 of the 282 tests.
[b]These combinations were available in 205 of the 282 tests.

3. Results

3.1. DP antibody frequencies

Positive reactions with DP-bearing beads were observed in 271 of the 650 sera. Most DP-reactive sera (62%) occurred in patients who had been previously transplanted, whereas the majority of DP-nonreactive (60%) sera occurred in patients with no previous transplants ($p < 0.0001$). There was no significant difference in the distribution of groups defined by race or gender among patients with or without DP-reactive antibody (Table 1).

Of the sera exhibiting reactivity with DP-bearing beads, the most frequently observed specificities were DP1 (54.6%), DP10 (54.3%), DP17 (51.8%), and DP13 (51.1%) (Fig. 1). The frequencies of DP15, DP18, and DP23 antibodies may be less representative because of their absence from some lots of the SA beads used. DP15 was present in 25 of 244 tests (10.2%), DP18 was present in 37 of 205 tests (18.0%), and DP23 was present in 32 of 205 tests (15.6%). The mean frequency of all DP antibodies was 38.9%. The frequencies of the different DP specificities were distributed evenly, with all except DP1 and DP10 falling within 1 standard deviation of the mean.

The frequencies of DP antibodies known to cross-react with DR1 or DR11 were determined as follows: (1) when the cross-reacting DR was present and (2) when the cross-reacting DR was absent, indicating that DP reactivity was caused by DP-specific antibody. These 2 groups were subdivided according to strength of reactivity: (1) weak positive, 500 to 1,000 MFI, (2) moderate positive, 1,000 to

Table 3
Donor mismatch mean fluorescence intensity (MFI) strengths for crossmatch tests

	Donor mismatch	Strength (MFI by single antigen)
Patient 1	DQ5	343
Titer > 512		
	DPB1*13:01	11,534
	DPB1*14:01	11,278
Patient 2	DR13	389
Titer >512		
	DQ6	1,793
	DPB1*02:01	18,208
Patient 3	DPB1*05:01	15,741 (at 1:8 dilution)
Titer = 4		
	DPB1*01:01	14,144 (at 1:8 dilution)

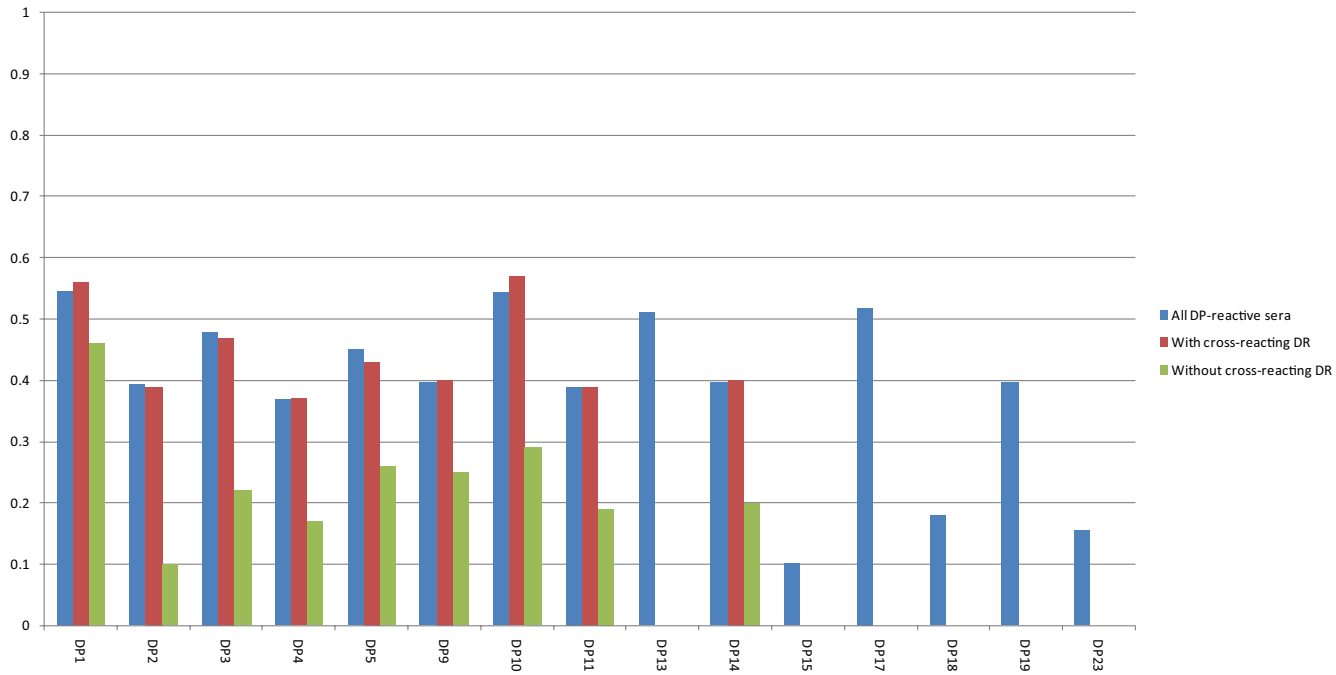

Fig. 1. Frequencies of DP-specific antibodies. The frequency of each DP specificity present in all sera positive for DP-specific antibody was calculated. The frequencies were then determined based on the presence or absence of DR-specific antibodies known to cross-react with the DP antibody. Note: DP13, DP15, DP17, DP18, DP19, and DP23 have not previously been demonstrated to be cross-reactive with any DR specificities.

5,000 MFI, and (3) strong positive, ≥5,000 MFI. We determined the mean MFI values for each DP specificity occurring in the presence and absence of the cross-reactive DR (Fig. 2). Only 58 of the 271 DP-reactive antibodies occurred in the absence of the cross-reacting DR antibody. Notably, 16 sera demonstrated reactivity only with DP and not with any other HLA antigen. The occurrence of DP antibodies in the absence of DR cross-reactivity decreased the DP antibody frequencies, as expected. The greatest decrease (39 to 10%) occurred with DP2-specific antibody (Fig. 3).

In almost all cases, the largest percentage (42.4%) of reactivity for DP was between 1,000 and 5,000 MFI. On average, 31.1% of the reactivity was between 500 and 1,000 MFI, 42.4% was between 1,000 and 5,000 MFI, and 26.4% was at ≥5,000 MFI. Of note, when antibody to DP4, DP15, or DP23 was observed, it was infrequently at strengths ≥5,000 MFI (13.5, 12.0, and 6.3%, respectively). In contrast, antibody to DP3 was observed often at strengths ≥5,000 MFI (40.7%). In most cases, the elimination of sera that exhibited cross-reactivity with DR antigens did not change the strength of the DP antibodies observed (Fig. 2).

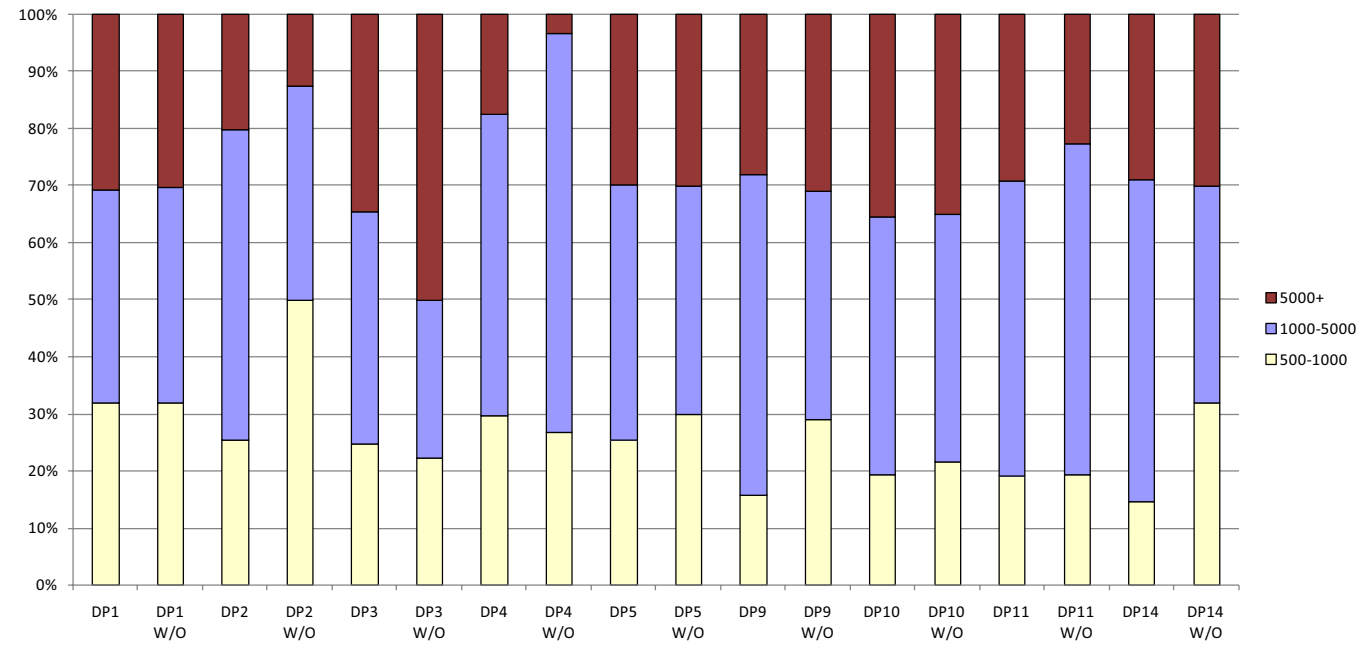

Fig. 2. Strength of DP antibody in the presence and absence of cross-reacting DR. The strength of those DP antigens known to share an epitope with DR antigens was examined in the presence and absence of those cross-reacting DR antibodies. In most cases, the presence or absence of cross-reacting DR antibodies did not affect the strength of the DP antibodies.

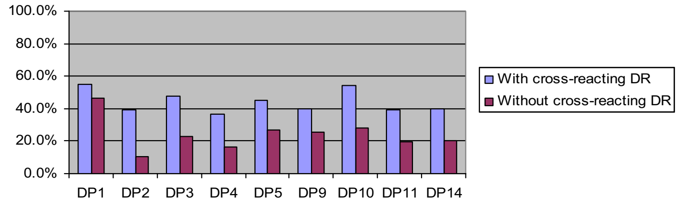

Fig. 3. Relative frequencies of DP-specific antibodies with versus without cross-reacting DR antibody. The frequencies of those DP antigens known to share an epitope with DR antigens were examined in the presence and absence of those cross-reacting DR antibodies. As expected, the frequency of all DP specificities was increased with the presence of the cross-reacting DR antibody.

3.2. Crossmatch tests

The 3 crossmatch tests yielded titers of 4 and >512 (2 tests). There were no non-DP targets to which the patient had antibody significant to yield a positive CDC crossmatch; therefore, the results for all 3 sera could only be accounted for by antibodies to the DP antigens of the target cell (Table 3).

3.3. DP serologic reactivity

Much of the observed DP reactivity was consistent with the DP epitopes described by Piazza et al. [14]. There were, however, sera for which the DP reactivity could not be explained on the basis of those epitopes. Researchers at the Johns Hopkins University Immunogenetics Laboratory and the MD Anderson Cancer Center identified 3 sera that appeared to display cross-reactivity not previously described. One of these sera was tested at both laboratories for confirmation. All 3 sera were reactive with DRB1*01:03 and DPB1*02:01, −09:01, −13:01, and −17:01, but not with DRB1*01:01, DRB1*01:02, or DRB1*11:01. One of the sera identified at the MD Anderson Cancer Center did exhibit some additional weak reactivity with DRB1*11:02, but the other 2 sera did not.

In an effort to identify amino acid substitutions in DPA and DPB molecules that could contribute to a putative epitope that would account for DP reactivity, sequence alignments were examined for various DP alleles, as well as for known DR alleles that cross-react with DP (DRB1*01:01 and DRB1*11:01) and the DRB1*01:03 that was seen reacting in the 3 identified sera (Fig. 4). A role for DPA1 polymorphism was ruled out because positive- and negative-reacting beads included the common alleles DPA1*01:03 and DPA1*02:01. Sequence alignments revealed a shared epitope that could contribute or potentially explain the observed cross-reactivity between DRB1*01:03 and the DPB1*02, −09, −10, −13, and −17 alleles. A shared ILE epitope was formed beginning at position 67 of the DRB1*01:03 sequence and position 65 of DPB1*02, −09, −10, −13, −16, and −17 sequences. This epitope is not present in either DRB1*01:01 (LLE) or DRB1*11:01 (FLE) sequences. All positive-reacting beads in the antibody analyses carried alleles with isoleucine (I) at residue 65 and glutamate (E) at residue 69. None of the negative-reacting beads included DPB1 alleles carrying both I and E at residues 65 and 69 simultaneously.

4. Discussion

In this study, we observed DP reactivity to be more frequent than previously reported. Approximately half of the patients with any HLA-specific antibody also demonstrated reactivity with DP. This finding is clinically significant because HLA-DP antibody has been associated with rejection [7–9]. Notably, sera reactive with DP were more likely to occur in previously transplanted patients than in patients who had not been transplanted (62 vs 38%). The frequency of DP reactivity did not differ among groups defined by race or gender. The nearly equal distribution of DP reactivity among men and women suggests that transplantation and transfusion, rather than pregnancy, are the major sources of sensitization to DP.

On the whole, the homogenous distribution of frequency and strength of the various DP antibodies suggest comparable immunogenicities. The significant reduction in frequency of DP-reacting antibodies in the absence of DR cross-reacting antibodies indicates that most DP antibodies are the result of cross-reactivity with DR (Fig. 2). However, DP reactivity is not completely dependent on sensitization to DR or to any HLA antigen, as indicated by the 16 sera (5.9% of sera with DP-specific antibody) demonstrating only DP reactivity. We observed that the absence of DR cross-reacting antibody decreases both the frequency and the strength of DP antibodies.

The crossmatch tests demonstrated that DP-specific antibody can be clinically relevant. All 3 crossmatch tests had positive results attributable only to DP. Additional crossmatch tests with selected cells are needed to determine whether differences in the strength of various DP specificities are a reflection of differences in immunogenicity, frequency of sensitization, or the composition of the beads in the solid-phase assay.

Three sera positive for DPB1*02, −09, −10, −13, −16, and −17 exhibited reactivity with DRB1*01:03, but not with DRB1*01:01 or DRB1*11:01. This pattern could not be explained by the previously identified epitopes, and sequence alignments of the involved alleles supported the new proposed cross-reactivity. Based on the allele sequences, if the DPB1 reactivity of these sera is defined by the recognition of a single structure, then the epitope identified must include I and E at residues 65 and 69. No other amino acid replacements at polymorphic residues 8, 9, 11, 35, 46, 55, 56, 57, 79, 84, 85, 86, or 87 are common to the DPB1 alleles 02:01, 09:01, 10:01, 13:01, 16:01, and 17:01.

In a similar analysis to the one performed by Samaniego et al. [8], we further examined the substitutions at equivalent DRB1 residues (I-67 and E-71). The DPB1 alleles included in the cross-reactive group that contain I65-E69 always have E at residue 68, whereas the DRB1 alleles that contain I67-E71 always have D at

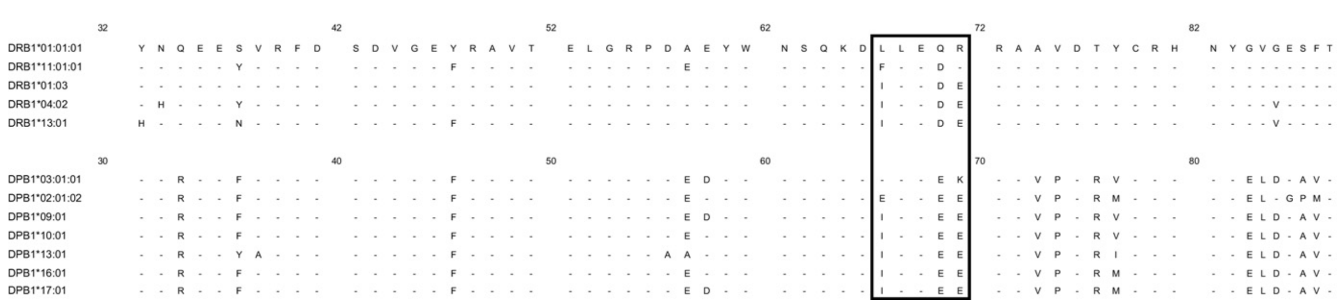

Fig. 4. Sequence alignment. DRB1 allele sequences are displayed starting at amino acid position 32, aligned with corresponding DPB1 allele sequences starting at position 30. The epitope shared between DRB1*01:03 and the DPB1*02, −09, −10, −13, and −17 alleles, suggested to be the cause of cross-reactivity, is seen in the boxed region. Other DRB1 and DPB1 sequences are also aligned to indicate the lack of this cross-reactive epitope, including DRB1*01:01 and DRB1*11:01, both of which are known to cross-react with certain DPB1 alleles.

residue 70. Importantly, the E-to-D amino acid difference in the sequence represents a conservative substitution because both are hydrophilic. The amino acid E/D at residues 60/70 of DPB1/DRB1, respectively, could play similar roles in defining a linear epitope; alternatively, the epitope specificity could be conferred by amino acid I at positions 65/67 and E at 69/71 of DRB1/DPB1 alleles, respectively. In 1 of the 3 sera identified exhibiting DRB1*01:03 cross-reactivity, reactivity with DRB1*13:01 was weak and was negative with DRB1*04:02. Both of these alleles carry the ILEDE linear sequence, as illustrated in Fig. 4. Therefore, there may be other regions of the DRB1 molecule that define the epitope by altering its conformation. One possibility is that whereas residues 65 to 69 or DPB1 and 67 to 71 of DRB1 define interlocus epitopes, other residues in these molecules may affect the conformation of the epitopes, resulting in differential recognition of the DRB1 alleles with the shared epitope. The epitope location on exon 2 is membrane distal and thus could be easily accessible by antibodies, suggesting that this shared epitope may be clinically significant. The putative epitope may be defined in part by the sequences IL(E/D)E spanning residues 65 to 69 of DPB1 or 67 to 71 of DRB1. The segment identified as helping to define the epitope includes residues E (glutamic) and D (aspartic) that have similar chemical characteristics because they are both acidic and negatively charged. The variable reactivity of the antibodies reactive with this epitope, among alleles of DRB1 bearing this amino acid stretch (DRB1*01:03, DRB1*04:02, DRB1*13:01, DRB1*13:02, DRB1*11:02), suggests that other variable segments in the DRB1 alleles may contribute to the structure of this epitope. We have been unable to map additional residues through a mere sequence comparison; it is possible that residues with similar physicochemical properties may define the binding characteristics of this epitope on DRB molecules.

Taken together, our data indicate a higher incidence of DP-specific antibodies than previously recognized, but not high as other HLA-specific antibodies. Importantly, sensitization to HLA-DP antigens may arise without direct exposure to DP mismatches through cross-reactivity with not only DRB1*01:01 and DRB1*1101, but also DRB1*01:03.

References

[1] Shaw S, Johnson AH, Shearer GM. Evidence for a new segregant series of B cell antigens that are encoded in the HLA-D region and that stimulate secondary allogenic proliferative and cytotoxic responses. J Exp Med 1980;152:565–80.
[2] Shaw S, DeMars R, Schlossman SF, Smith PL, Lampson LA, Nadler LM. Serologic identification of the human secondary B cell antigens. Correlations between function, genetics, and structure. J Exp Med 1982;156:731–43.
[3] Youngs D. HLA-DP alloantibodies. ASHI Q 2004;28:60.
[4] Goral S, Prak EL, Kearns J, et al. Preformed donor-directed anti-HLA-DP antibodies may be an impediment to successful kidney transplantation. Nephrol Dial Transplant 2008;23:390–2.
[5] Tambur AR, Buckingham M, McDonald L, Luo X. Development of donor-specific and non-donor-specific HLA-DP antibodies post-transplant: the role of epitope sharing and epitope matching. Clin Transpl 2006:399–404.
[6] Mucynski KA, Ekle DM, Coder DM, Anderson SK. Normal human kidney HLA-DR-expressing renal microvascular endothelial cells: characterization, isolation, and regulation of MHC class II expression. J Am Soc Nephrol 2003;14:1336–48.
[7] Thaunat O, Hanf W, Dubois V, et al. Chronic humoral rejection mediated by anti-HLA-DP alloantibodies: insights into the role of epitope sharing in donor-specific and non-donor specific alloantibodies generation. Transpl Immunol 2009;20:209–11.
[8] Samaniego M, Mezrich J, Torrealba J, et al. C4d-positive acute antibody-mediated rejection due to anti-HLA-DP antibody: a tale of one patient and a review of the University of Wisconsin experience. Clin Transpl 2006;503–7.
[9] Singh P, Colombe BW, Francos GC, Martinez Cantarin MP, Frank AM. Acute humoral rejection in a zero mismatch deceased donor renal transplant due to an antibody to an HLA-DP alpha. Transplantation 2010;90:220–1.
[10] Qiu J, Cai J, Terasaki PI, El-Awar N, Lee JH. Detection of antibodies to HLA-DP in renal transplant recipients using single antigen beads. Transplantation 2005;80:1511–3.
[11] Duquesnoy RJ, Awadalla Y, Lomago J, et al. Retransplant candidates have donor-specific antibodies that react with structurally defined HLA-DR, DQ, DP epitopes. Transpl Immunol 2008;8:352–60.
[12] Cano P, Fernandez-Vina M. Two sequence dimorphisms of DPB1 define the immunodominant serologic epitopes of HLA-DP. Hum Immunol 2009;70:836–43.
[13] Zachary AA, Sholander JT, Houp JA, Leffell MS. Using real data for a virtual crossmatch. Hum Immunol 2009;70:574–9.
[14] Piazza A, Poggi E, Ozzella G, et al. Anti-HLA-DP antibodies and graft failure in kidney transplantation. Transplantation 2006;82:242.