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Two sequence dimorphisms of DPB1 define the immunodominant serologic epitopes of HLA-DP

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ABSTRACT

We describe two sets of epitopes present in HLA-DP molecules; they were identified with alloantibodies from clinical serum samples. Specificity was determined using fluorescent beads coated with single antigens and detected in a Luminex platform. Of the patients with anti–HLA class II antibodies, 18% had anti-DP antibodies; among these, 24 of 32 patients (75%) had antibodies against the dimorphic epitope sets described here. Residues 56-A and 56-E divide DPB1 alleles into two mutually exclusive and collectively exhaustive groups. These groups have distinctive dimorphic epitopes that are detected by antibodies. Epitope P-001, identified by 2 sera, is defined by residue 56-A of the DPB subunit. Epitope P-002, identified by 9 sera, is defined by residue 56-E. Interlocus DRB1/DPB1 reactivity is associated with P-002, which is found in DRB1–DR11 alleles. Residues at DPB1 85-87-EAV define the P-003 epitope, whereas P-004 is defined by 85–87-GPM. This dimorphism also divides DPB1 alleles into two mutually exclusive and collectively exhaustive groups. In this study, 12 patient sera identified DP-003, and 1 identified DP-004. Two dimorphic systems account largely for the serologic features of the DP molecules, and these specificities were found in most clinical samples with anti-DP activity.

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1. Introduction

HLA-DP antigen–antibody reactivity is likely to play an important role in both solid-organ transplantation and bone marrow transplantation. Examples of the value of studies of anti-DP antibodies are shown in the characterization of DP serologic determinants described in the following two cases.

A patient with end-stage renal disease waiting for a renal transplant has a positive antibody screen with broad specificity against many antigens in HLA-B, C, DR, and DQ. A kidney becomes available matching at HLA-A, B, C, DR, and DQ with the patient. A T-cell flow crossmatch is negative, and a B-cell flow crossmatch is positive. Further analysis of the serum reveals the presence of anti-DP antibodies reacting with single-antigen beads carrying DPB1*1701, 1001, 0301, and 0201, and does not react with DPB1*0101, 0401, 0501 and 1301. The patient's phenotype is DPB1*0401/0501 and the donor's is DPB1*0501/0601. The patient does not undergo transplantation [1].

A patient with AML is refractory to chemotherapy. An unrelated donor is found matching at HLA-A, B, C, DRB1, DRB3/4/5, and DQB1, with a mismatch in DPB1. The patient has DPB1*0401/1501 and the donor has DPB1*0301/1501. An antibody screen shows the presence of anti-HLA antibodies with specificity against multiple antigens in HLA-A, B, C, DQ, and DP. The anti-DP antibodies react against single-antigen beads with DRB1*1701, 0101, 0501, 1001, 1401, 0301, 1901, 0901, and 1301, and failing to react with 0201, 1101, and 0401. Despite the presence of anti-DPB1*0301, a transplantation was performed with the bone marrow of this donor. Four weeks after transplantation there are no signs of engraftment. The intensity of the antibody decreases to one fifth of the original reactivity before transplantation. Plasmapheresis is then performed, and the anti-DP antibody becomes undetectable. A second transplantation from the same donor is carried out. Four weeks after the second transplantation, chimerism studies show signs of engraftment.

To define anti-DP reactivity in a patient's serum and to predict reactivity with a specific DP antigen, it is essential to characterize the epitopes present in DP heterodimers. Ideally, the serologic reactivity data should be interpreted in terms of well-characterized serologic epitopes defined by the amino acid sequence properties of those alleles that carry the epitopes. The anti-HLA antibody specificities of a serum specimen could then be reported as the epitopes that it recognizes and not by a list of traditional serologic specificities or even a list of alleles.

The use of epitopes, instead of traditional serologic specificities, has the following advantages: 1) it provides a coherent summary of the serologic reactivity of a particular serum sample; 2) it precisely and accurately predicts reactivity not only with those alleles present in the reagents used for testing, but also with all other alleles not included in the reagents; and 3) it provides the theoret-

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ical foundation for solving a difficult practical problem in antibody identification: setting the cutoff point used for distinguishing positive from negative results. A comprehensive catalog of epitopes will allow the investigation of the potential differences in immunogenicity. The knowledge gained from these studies could then be introduced in organ allocation algorithms that could result in better transplantation outcomes.

A serologic epitope can be defined as the physical area of an antigenic molecule that an antibody binds to in a specific way. In the case of proteins, epitopes are defined by the tertiary conformation of amino acid sequences. Insofar as the tertiary conformation of a protein is determined by the primary sequence of amino acids, epitopes can be defined in terms of this primary sequence of amino acids. In many examples, the consecutive linear sequence of amino acids in the primary structure of a protein does not necessary define an epitope. It is possible for amino acids distant from each other in the primary structure to be close in the tertiary structure. It is also possible that amino acids away from the physical antigen–antibody interaction area contribute to the definition of the epitope by affecting the tertiary structure of the protein.

It is clear that the entire molecule is required to physically determine an epitope, but when it comes to defining an epitope in logical terms, the principle of parsimony comes into effect. The logical definition of an epitopes requires the following steps. 1) Identify serum samples with single antibody specificity, or isolate single antibodies from serum samples by means of adsorptions. 2) Classify alleles into two classes: alleles that react with the antibody in question, and alleles that fail to react. 3) Map the empirical classification in step 2 to the distinct sequence properties of the alleles in the two classes. This mapping can take the form of the following question: Which sequence properties are present in one class and absent in the other?

This mapping can be comprehensive, including all of the sequence properties that distinguish the two classes; or it can be minimalist, including just the smallest number of properties necessary to distinguish the two classes. The minimalist approach can result in multiple possible mappings. It must be kept in mind that there can be multiple, effective ways to define an epitope in terms of amino acid sequences properties.

In regard to the cutoff point problem in antibody identification, a distinction must be made between general reactivity cutoff points to assess antigen–antibody reactivity in regard to its clinical significance, on one hand; and specific sample reactivity cutoff points to assess whether there is a reaction, regardless of whether it is clinically significant, on the other hand. The first general cutoff point is used among multiple samples to predict clinical relevance. Single-antigen bead reactivity is a poor predictor of clinical relevance, and it would be imprudent to set a definitive cutoff point to make clinical decisions based on it. The second sense in which we talk about cutoff points refers to individual samples and the individual bead reactivity in that sample that should be taken as positive or negative. This has nothing to do with clinical relevance. It is all about discriminating between true reactivity and background noise. These sample reactivity cutoff points are used to evaluate the specificity of the antibodies. In talking about cutoff points in this sense, allowed cutoff points are not continuous in the set of reactivity values, but jump from one level to another in a discrete fashion: only those cutoff points consistent with the definition of known epitopes are allowed. In addition, of course, cutoff points must be set above reactivity levels with self antigens. For this reason it is necessary to know the HLA type of a subject to interpret the reactivity of antibodies present in the serum from that subject.

2. Subjects and methods

Of 1400 patients evaluated as prospective candidates for bone marrow transplantation, 173 had anti–HLA class II antibodies. Of these 173 patients, 32 had anti-DP antibodies. Sera from these 32 patients were used in this study.

The specificity of anti–HLA-DP antibodies present in clinical serum samples was characterized with fluorescent beads coated with single antigens (One Lambda, Canoga Park, CA) and detected in a Luminex platform. The single-antigen beads used for DP antibody characterization included the following DPB1 alleles: DPB1*0101, 0201, 0301, 0401, 0402, 0501, 0901, 1001, 1101, 1301, 1401, 1701, and 1901; these were used to test all sera. Each single-antigen DP molecule that contained these DPB1 alleles included heterodimers with DPA1 alleles that correspond to the most frequent DPA1–DPB1 associations in European populations. A limitation of this panel is the lack of representation of less common DPA1–DPB1 associations that limited the identification of antibodies specific only for some DPA1 alleles. Some sera were tested with kits that contained additional DP beads carrying DPB1*0202, 1501, 1801, and 2301; the inclusion of the latter antigens helped in the identification of DPA1-associated reactivity.

The Pearson correlation coefficient between all pairs of single-antigen bead reactivity was calculated and used as a similarity measure to analyze the presence of clusters. These clusters of antigens or alleles were assumed to form by bearing a common feature that was identified by antibodies; we postulated them to be the epitopes. Alleles in one cluster were proposed to share an epitope. Allele sequences were then compared with search for unique sequence properties that would distinguish alleles in one cluster from those outside the cluster.

In measuring reactivity for a set of beads, the maximum value for the set was considered more representative than average. The logic for this was based on the assumption that only antigen–antibody reactivity can be responsible for strong reactions, whereas these reactions can be weakened by a number of factors including variations in technical performance, variable amounts of antigen in different beads, and contributions by variations in either the DPA subunit or other segments of the molecule. Some kind of standardization, taking into account variation between runs, is necessary.

One-way analysis of variance (ANOVA) was performed on different antibodies identified in clinical samples, to compare the reactivity with different epitopes.

The final assignment of the antibody reactivity of serum was defined in terms of the reactivity with single-antigen beads and grouped according to the putative epitopes that it may recognize.

Once the primary DP serologic groups were identified, a relational data model was created to store Luminex values and the specificity of single antigens on the beads. The data were managed using SQL to adjust the cutoff point in calling a positive or negative reaction. Contingency tables (2×2) were created for each combination of epitopes on the single antigens present on the beads. A computer program was created to identify the optimal cutoff point for each sample by minimizing the number of false-positive and false-negative values on the 2×2 contingency tables.

3. Results

In looking at the descriptive statistics of the bead reactivity with serum samples carrying anti-DP antibodies, it became clear that the antigens with lower total average reactivity were the most prevalent in the general population from which the samples came. DPB1*0401 showed a mean reactivity of 607, and DPB1*0402, a mean reactivity of 581, when all the other beads showed average reactivities ranging between 1011 and 2951. We reasoned that the more frequent an allele is found in a population, the less likely it is to be identified by an antibody against it in that population.

Figure 1 shows a similarity matrix between the single antigens on the beads based on the Pearson correlation coefficient between the raw values of antibody reactivity and these beads. The single

ALL 68 CASES

Correlations: 0101, 0301, 0501, 0901, 1001, 1301, 1401, 1701, 1901, 1101, 0401,

P003 (cluster: 0101, 0301, 0501, 0901, 1001, 1301, 1401, 1701, 1901, 1101) — P004 (cluster: 0401, 0402, 0201)

	0101	0301	0501	0901	1001	1301	1401	1701	1901	1101	0401	0402
0301	0.610											
	0.000											
0501	0.987	0.655										
	0.000	0.000										
0901	0.756	0.877	0.803									
	0.000	0.000	0.000									
1001	0.650	0.790	0.675	0.871								
	0.000	0.000	0.000	0.000								
1301	0.884	0.660	0.889	0.783	0.613							
	0.000	0.000	0.000	0.000	0.000							
1401	0.755	0.879	0.795	0.988	0.871	0.771						
	0.000	0.000	0.000	0.000	0.000	0.000						
1701	0.716	0.772	0.763	0.825	0.773	0.586	0.795					
	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
1901	0.680	0.714	0.688	0.798	0.665	0.764	0.820	0.512				
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
1101	0.547	0.652	0.558	0.774	0.619	0.717	0.797	0.362	0.922			
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000			
0401	0.013	-0.024	-0.004	0.205	0.152	0.143	0.202	-0.089	0.511	0.574		
	0.918	0.843	0.973	0.093	0.216	0.243	0.098	0.471	0.000	0.000		
0402	-0.149	0.193	-0.117	0.349	0.395	0.022	0.340	0.074	0.380	0.517	0.778	
	0.226	0.116	0.342	0.004	0.001	0.862	0.005	0.548	0.001	0.000	0.000	
0201	-0.247	0.137	-0.234	0.179	0.436	-0.114	0.161	0.072	0.152	0.216	0.550	0.825
	0.042	0.266	0.055	0.144	0.000	0.357	0.190	0.557	0.217	0.077	0.000	0.000

Cell Contents: Pearson correlation
P-Value

Fig. 1. Cluster analysis based on the Pearson correlation coefficients between the mean reactivity of each pair of single-antigen beads (all cases included).

antigens were rearranged in the matrix, as shown in this figure, using a clustering algorithm. All reactions were included in this comparison. This figure shows two distinct clusters: one including DPB1*0101, 0301, 0501, 0901, 1001, 1301, 1401, 1701, 1901, and 1101, and the other cluster including DPB1*0401, 0402, and 0201.

Figure 2 shows how the previous serologic reactivity dichotomy can be explained in terms of amino acid sequence differences at positions 85–87.

Again, because of the prevalence of allele DPB1*0401 in the population and the corresponding epitope defined by amino-acid residues at positions 85–87, antibodies against the other corresponding epitope must, by necessity, also be the most common antibody, which could hide other antibodies. By removing the cases with reactivity patterns consistent with this antibody, we repeated the cluster analysis shown in Fig. 2 with the remaining cases.

Figure 3 shows a second similarity matrix between single antigens based on the Pearson correlation coefficients between raw values of antibody reactivity. Cluster analysis shows a new set of two different clusters different from those shown in Fig. 2. The DPB1 alleles cluster in these two groups are as follows: a) one including DPB1*0101, 0401, 0501, 1101, 1301, and 1901; and b) one including DPB1*0201, 0301, 0402, 0901, 1001, 1401, and 1701.

First observed dimorphism in serologic reactivity is explained by amino acid residues at positions 85-87

```
                    10         20         30         40         50         60         70         80         90        100
DPB1*010101  RATPENYVYQ GRQECYAFNG TQRFLERYIY NREEYARFDS DVGEFRAVTE LGRPAAEYWN SQKDILEEKR AVPDRVCRHN YELDEAVTLQ RRVQPKVNVS
DPB1*020102  RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEEYWN SQKDILEEER AVPDRMCRHN YELGGPMTLQ RRVQPRVNVS
DPB1*030101  RATPENYVYQ LRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEDYWN SQKDLLEEKR AVPDRVCRHN YELDEAVTLQ RRVQPKVNVS
DPB1*040101  RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFARFDS DVGEFRAVTE LGRPAAEYWN SQKDILEEKR AVPDRMCRHN YELGGPMTLQ RRVQPRVNVS
DPB1*0402    RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEEYWN SQKDILEEKR AVPDRMCRHN YELGGPMTLQ RRVQPRVNVS
DPB1*0501    RATPENYLFQ GRQECYAFNG TQRFLERYIY NREELVRFDS DVGEFRAVTE LGRPEAEYWN SQKDILEEKR AVPDRMCRHN YELDEAVTLQ RRVQPKVNVS
DPB1*0901    RATPENYVHQ LRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEDYWN SQKDILEEER AVPDRVCRHN YELDEAVTLQ RRVQPKVNVS
DPB1*1001    *****NYVHQ LRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEEYWN SQKDILEEER AVPDRVCRHN YELDEAVTLQ RR********
DPB1*110101  *****NYVYQ LRQECYAFNG TQRFLERYIY NRQEYARFDS DVGEFRAVTE LGRPAAEYWN SQKDLLEERR AVPDRMCRHN YELDEAVTLQ RR********
DPB1*1301    RATPENYVYQ LRQECYAFNG TQRFLERYIY NREEYARFDS DVGEFRAVTE LGRPAAEYWN SQKDILEEER AVPDRICRHN YELDEAVTLQ RRVQPKVNVS
DPB1*1401    RATPENYVHQ LRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEDYWN SQKDLLEEKR AVPDRVCRHN YELDEAVTLQ RRVQPKVNVS
DPB1*1701    *****NYVHQ LRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEDYWN SQKDILEEER AVPDRMCRHN YELDEAVTLQ RR********
DPB1*1901    RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPEAEYWN SQKDILEEER AVPDRICRHN YELDEAVTLQ RRVQPKVNVS
```

Fig. 2. Amino acid sequence differences between the two clusters of DPB1 alleles identified in Fig. 1.

11 SELECTED CASES WITH DISTINCT REACTIVITY PATTERN

P001

Correlations: 0101, 0401, 0501, 1101, 1301, 1901, 0201, 0301, 0402, 0901, 1001,

	0101	0401	0501	1101	1301	1901	0201	0301	0402	0901	1001	1401
0401	0.995 0.000											
0501	0.997 0.000	1.000 0.000										
1101	0.999 0.000	0.995 0.000	0.997 0.000									
1301	0.996 0.000	0.990 0.000	0.991 0.000	0.993 0.000								
1901	0.997 0.000	0.997 0.000	0.998 0.000	0.996 0.000	0.997 0.000							
0201	-0.633 0.036	-0.621 0.041	-0.618 0.043	-0.620 0.042	-0.652 0.030	-0.630 0.038						
0301	-0.474 0.140	-0.458 0.156	-0.451 0.164	-0.449 0.166	-0.478 0.137	-0.456 0.159	0.846 0.001					
0402	-0.423 0.195	-0.407 0.214	-0.402 0.221	-0.402 0.220	-0.439 0.176	-0.414 0.205	0.952 0.000	0.887 0.000				
0901	-0.431 0.186	-0.415 0.204	-0.408 0.213	-0.404 0.217	-0.432 0.184	-0.411 0.210	0.792 0.004	0.991 0.000	0.838 0.001			
1001	-0.635 0.036	-0.620 0.042	-0.617 0.043	-0.621 0.042	-0.659 0.027	-0.636 0.036	0.989 0.000	0.829 0.002	0.928 0.000	0.782 0.004		
1401	-0.444 0.172	-0.429 0.188	-0.422 0.196	-0.418 0.201	-0.442 0.173	-0.421 0.198	0.785 0.004	0.989 0.000	0.826 0.002	0.996 0.000	0.766 0.006	
1701	-0.513 0.107	-0.495 0.122	-0.488 0.128	-0.485 0.130	-0.521 0.101	-0.491 0.126	0.820 0.002	0.972 0.000	0.838 0.001	0.966 0.000	0.796 0.003	0.978 0.000

P004

Cell Contents: Pearson correlation
P-Value

Fig. 3. Cluster analysis based on the Pearson correlation coefficients between the mean reactivity of each pair of single-antigen beads. Cases with anti-P003, the most frequent antibody, have been removed to show what *else* remains.

Figure 4 shows this new serologic reactivity dichotomy explained this time in terms of the amino acid residues at position 56.

This study presents two dimorphic epitope sets present in HLA-DP. We observed that 32 of 178 (18%) of subjects with anti-HLA class II antibodies have anti-DP antibodies. Of the 32 subjects with anti-DP antibodies, 24 (75%) show clear specificity against the dimorphic epitope sets described here. The rest of the cases have weak reactivity, and the presence and absence of antigen–antibody reactivity is difficult to evaluate with precision.

Two dimorphic epitope systems were identified, as described below.

3.1. *P-001/P-002 epitope set*

Residues 56-A and 56-E divide DPB1 alleles into two mutually exclusive and collectively exhaustive groups. These groups have distinct dimorphic epitopes that are detected by antibodies.

Second observed dimorphism is serologic reactivity is explained by amino acid residue at position 56.

```
                      10         20         30         40         50         60         70         80         90        100
DPB1*010101   RATPENYVYQ GRQECYAFNG TQRFLERYIY NREEYARFDS DVGEFRAVTE LGRPAAEYWN SQKDILEEKR AVPDRVCRHN YELDEAVTLQ RRVQPKVNVS
DPB1*020102   RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEEYWN SQKDILEEER AVPDRMCRHN YELGGPMTLQ RRVQPRVNVS
DPB1*030101   RATPENYVYQ LRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEDYWN SQKDLLEEKR AVPDRVCRHN YELDEAVTLQ RRVQPKVNVS
DPB1*040101   RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFARFDS DVGEFRAVTE LGRPAAEYWN SQKDILEEKR AVPDRMCRHN YELGGPMTLQ RRVQPRVNVS
DPB1*0402     RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEEYWN SQKDILEEKR AVPDRMCRHN YELGGPMTLQ RRVQPRVNVS
DPB1*0501     RATPENYLFQ GRQECYAFNG TQRFLERYIY NREELVRFDS DVGEFRAVTE LGRPEAEYWN SQKDILEEKR AVPDRMCRHN YELDEAVTLQ RRVQPKVNVS
DPB1*0901     RATPENYVHQ LRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEDYWN SQKDILEEER AVPDRVCRHN YELDEAVTLQ RRVQPKVNVS
DPB1*1001     *****NYVHQ LRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEEYWN SQKDILEEER AVPDRVCRHN YELDEAVTLQ RR********
DPB1*110101   *****NYVYQ LRQECYAFNG TQRFLERYIY NRQEYARFDS DVGEFRAVTE LGRPAAEYWN SQKDLLEERR AVPDRMCRHN YELDEAVTLQ RR********
DPB1*1301     RATPENYVYQ LRQECYAFNG TQRFLERYIY NREEYARFDS DVGEFRAVTE LGRPAAEYWN SQKDILEEER AVPDRICRHN YELDEAVTLQ RRVQPKVNVS
DPB1*1401     RATPENYVHQ LRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEDYWN SQKDLLEEKR AVPDRVCRHN YELDEAVTLQ RRVQPKVNVS
DPB1*1701     *****NYVHQ LRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPDEDYWN SQKDILEEER AVPDRMCRHN YELDEAVTLQ RR********
DPB1*1901     RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFVRFDS DVGEFRAVTE LGRPEAEYWN SQKDILEEER AVPDRICRHN YELDEAVTLQ RRVQPKVNVS
```

Fig. 4. Amino acid sequence differences between the two clusters of DPB1 alleles identified in Fig. 3.

Epitope P-001 is defined by residue 56-A and epitope P-002 by residue 56-E. Anti–P-002 also recognizes DR11 alleles, as they share that epitope with DPB1 alleles, although the sequences are shifted two positions: 56-E in DPB1 is 58-E in DRB1. Two patients had anti–P-001 and nine had anti–P-002. The nine patients with anti-P-002 had sera that always reacted with DR11. P-002 was originally detected by monoclonal antibodies called "ILR1" [2]. The crossreactivity between some DP molecules bearing the glutamate at residue 56 of the DPB subunit with DR11 molecules was earlier noticed by Bodmer *et al.* in 1987 [2] and further confirmed by Klohe *et al.* in 1992 [3].

3.2. P-003/P-004 epitope set

Residues 85-E, 86-A, and 87-V define the P-003 epitope. Residues 85-G, 86-P, and 87-M define the P-004. These two epitopes also divide DPB1 alleles into two mutually exclusive and collectively exhaustive groups. Twelve patients had anti–P-003. One patient had anti-P-004, although mixed with anti-DPA1*0201 reactivity. P-003 and P-004 were originally detected by monoclonal antibodies called "WT" and "BUT" respectively [4]. P-004 was also called "DP11.1" [2].

According to the two dimorphic systems described above, each and every DPB1 allele can be classified into four primary serologic groups that we denominate DP1, DP2, DP3, or DP4, based on the presence or absence of the P-001/002 and P-003/004 epitopes, and characterized by the amino acid residues at position 56 and 85–87. Interestingly, these serologic types distinguish the first four original primed lymphocyte typing (PLT)-defined specificities SB1, SB2, SB3, and SB4 that correspond to the alleles DPB1*0101, 0201, 0301, and 0401, respectively (Figs. 5 and 6).

We calculated the correlation coefficients between the reactivity of clinical sera with single-antigen beads grouped not according to the allele name but according to the serologic group of the allele as just defined. These correlation coefficients show empirically that the following pairs of serologic groups have something in common: DP1 and DP3 ($r^2 = 0.7$, $p < 0.001$), DP1 and DP4 ($r^2 = 0.4$, $p = 0.001$), DP2 and DP3 ($r^2 = 0.4$, $p = 0.001$), and DP2 and DP4 ($r^2 = 0.4$, $p < 0.001$). It can be postulated that what each pair has in common is the presence of a shared epitope. At the same time, the following pairs do not have any common epitope: DP1 and DP2 ($r^2 = 0.1$, $p = 0.402$), and DP3 and DP4 ($r^2 = 0.0$, $p = 0.871$). Figure 6 represents the epitopes present in each of the serologic groups.

We used one-way analysis of variance of the reactivity patterns on the epitopes carried by the beads in cases with anti-P001, anti-P002, and anti-P003 to see the difference in reactivity by the epitope carried by each bead. For each sample the maximum reactivity for all the beads carrying an epitope was calculated, and then the mean of all of the samples maximum reactivity per epitope was calculated. The anti-P001 sera showed a mean reactivity of 4760 for the beads carrying the P001 epitope and 253 for the beads carrying the P002 epitope ($p < 0.001$). The anti-P002 sera showed a mean reactivity of 198 for the beads carrying the P001 epitope and 3308 for the beads carrying the P002 epitope ($p < 0.001$). The anti-P003 sera showed a mean reactivity of 4457 for the beads carrying the P003 epitope and 271 for the beads carrying the P004 epitope ($p < 0.001$). If the bead reactivity is expressed in terms of the epitopes present on the bead, the specificity of the antibody becomes clearly exposed.

In the analysis of the DPB1 alleles in patients making anti-DP antibodies, we consistently observed the absence of the epitope in question in the patients who produced the antibodies against that epitope. Knowledge of the HLA-DPA1 and DPB1 phenotypes provides essential information for the evaluation of the specificity of the antibody.

	P-001/002 P:56	P-003/004 P:85-87	
DPB1*010101	A	EAV	DP1
DPB1*010102	A	EAV	
DPB1*0302	A	EAV	
DPB1*0501	A	EAV	
DPB1*110101	A	EAV	
DPB1*1301	A	EAV	
DPB1*1901	A	EAV	
DPB1*2101	A	EAV	
DPB1*2201	A	EAV	
DPB1*260101	A	EAV	
DPB1*260102	A	EAV	
DPB1*2701	A	EAV	
DPB1*3001	A	EAV	
DPB1*3101	A	EAV	
DPB1*3601	A	EAV	
DPB1*3801	A	EAV	
DPB1*5501	A	EAV	
DPB1*6301	A	EAV	
DPB1*8501	A	EAV	
DPB1*0202	A	GPM	DP4
DPB1*040101	A	GPM	
DPB1*1501	A	GPM	
DPB1*2301	A	GPM	
DPB1*3301	A	GPM	
DPB1*3401	A	GPM	
DPB1*3901	A	GPM	
DPB1*4001	A	GPM	
DPB1*4701	A	GPM	
DPB1*7201	A	GPM	
DPB1*030101	E	EAV	DP3
DPB1*0601	E	EAV	
DPB1*0901	E	EAV	
DPB1*1001	E	EAV	
DPB1*1401	E	EAV	
DPB1*1601	E	EAV	
DPB1*1701	E	EAV	
DPB1*200101	E	EAV	
DPB1*2901	E	EAV	
DPB1*3501	E	EAV	
DPB1*4501	E	EAV	
DPB1*5001	E	EAV	
DPB1*6901	E	EAV	
DPB1*7801	E	EAV	
DPB1*020102	E	GPM	DP2
DPB1*0402	E	GPM	
DPB1*0602	E	GPM	
DPB1*1801	E	GPM	
DPB1*2801	E	GPM	
DPB1*4601	E	GPM	
DPB1*4801	E	GPM	
DPB1*4901	E	GPM	
DPB1*5101	E	GPM	
DPB1*5901	E	GPM	
DPB1*8101	E	GPM	

Fig. 5. Each well-documented DPB1 allele can be classified into four primary serologic groups: DP1, DP2, DP3, or DP4, based on the presence or absence of the P-001/P-002 and P-003/P-004 epitopes.

Epitopes	DP1	DP2	DP3	DP4
P-001				
P-002				
P-003				
P-004				

Fig. 6. Primary DPB1 serologic types, which coincide with the first four original PLT-defined specificities SB1, SB2, SB3, and SB4.

A preliminary analysis appears to indicate that DPA1 alleles may correlate with the intensity of the binding between antibodies and beads bearing the epitopes present in the DPB subunits; however, it appears that in all cases described in the present study, the specificities defined by two dimorphic epitope systems presented here are preserved independently of the DPA1 alleles. There are less common cases showing sera with anti-DP reactivity that can be accounted for only in terms of the DPA1 chain and not the DPB1 chain. We identified three sera in which reactivity against DPA1*0201 could be invoked, although, in addition, the reactivity of these sera was completely consistent with the presence of antibodies reactive with anti-P-001 and anti-P-004 [2, 4-6].

4. Discussion

This study shows that in sera from patients waiting for an allotransplant, two dimorphic epitope systems account for a large proportion of the serologic reactivity with DP molecules. Several of these epitopes were originally characterized more than 20 years ago with monoclonal antibodies and correspond exactly with the specificity observed in the great majority of antibodies detected in clinical samples.

Each and every DPB1 allele carries two epitopes: either P-001 or P-002 on one hand, and either P-003 or P-004 on the other hand. This allows the classification of DPB1 alleles into four primary serologic groups: DP1, DP2, DP3, or DP4. The DP1 serologic group carries the P-001 and the P-003 epitopes; DP2 carries P-002 and P-004; DP3 carries P-002 and P-003; and DP4 carries the P-001 and P-004 epitopes (Fig. 5). It is possible to obtain sera with anti-P-001, anti-P-002, anti-P-003, and anti-P-004 reactivity, which could theoretically be used to type DPB1 alleles serologically and to classify them into the four primary serologic groups. We found samples that carried all of these serologic specificities in pure form, except for anti-P-004, which, because of the prevalence of DPB1*0401, is difficult to find. This serologic behavior of DPB1 alleles can be characterized and predicted by the amino acid residues at positions 56 and 85–87 in each allele.

The probability of antigen exposure to P-004 is 0.06 in European, 0.16 in African, and 0.22 in Asian populations; these probabilities were calculated from studies conducted in several ethnic groups (M. Fernández-Viña, personal communication). Therefore, the fact that European descent is predominant in our patients explains why we have not seen more cases of anti–P-004. It is approximately three to five times more likely that an anti–P-004 antibody will be found in patients of African or Asian ancestry than in patients of European ancestry. However, we did see a case of anti–P-004 mixed anti-DPA1 reactivity. Bodmer *et al.* produced a xenogeneic monoclonal antibody with this specificity more than 20 years ago [2]. In contrast, the probability of antigen exposure to the alternative epitope, P-003, is 0.25 in Europeans, four times higher than that of P-004, against which we have seen antibodies in 12 patients.

It is possible that there are differences in immunogenicity that account for deviations from expectations of antibody frequency based on probability of antigen exposure. It should be noted that this may depend on both the structure of the epitope and on antigen density. In the case of P-002 and P-001, the former epitope is expressed on the high-density HLA-DR11 molecule as well as in DP molecules, whereas the latter epitope appears to be found only in DP molecules. We have seen nine cases of anti–P-002 and only two patients with reactivity against P-001. More cases would be required to accurately assess immunogenicity. Modeled after HLA-DR, and correcting for the two–amino acid shift between the two loci, it can predicted that residue 56 and residues 85–87 are located in the alpha helical segments of the beta subunit and are directly assessable to antibodies and to the T-cell receptor.

Like the Bw4/Bw6 serologic dimorphism that classifies HLA-B alleles into two mutually exclusive and collectively exhaustive groups, the P-001/P-002 dimorphism separates DPB1 alleles into two distinct groups detected by clinical sera. Also, just like the anti-Bw4 antibodies that detect alleles at another locus (HLA-A23, A24, A25 and A32), the anti–P-002 antibodies present reactivity against some alleles of HLA-DR (encoded by DRB1*11 alleles) in addition to the anti-DP reactivity. The characterization of HLA epitopes is essential in the interpretation of anti-HLA antibody reactivity. It reveals the structure against which the antibodies present in a serum react; it can also provide a detailed listing of the individual alleles that may react with a given serum in spite of not having been tested directly. Rather than considering the list of individual specificities as the result of a random event, it may also be useful to predict interactions with alleles in other loci, and it is the key to establish cutoff points for the intensity signal of antigen–antibody reactivity.

We identified sera from eight patients with anti-DP reactivity; their specificity could not be accounted for in terms of the two dimorphism described here. These sera showed reactivity that might be explained in terms of other less common or less immunogenic DP epitopes. The putative additional epitopes could include 55-D-56-E-57-D (P-005), 55-D-56-E-57-E (P-006), and 65-I-66-L-67-E-68-E-69-E (P-007), as well as DPA1 epitopes. Goral *et al.* discuss the role of anti-DP antibodies in kidney transplantation and identify a patient with anti–P-005 [7].

A principle of immunodominance is in effect here. P-001, P-002, P-003, and P-004 are the immunodominant epitopes in DP serology. Patients who lack any of these epitopes will predominantly tend to develop antibodies against them rather than the other nonimmunodominant epitopes such as P-005, P-006, and P-007. Only when a patient carries all of the immunodominant epitopes will that patient be predisposed to develop antibodies against non-immunodominant epitopes.

In 2005, Arnold *at al.* identified some DPB1 epitopes that explained the antibody reactivity that they observed. They found 14 cases with anti–P-002 reactivity and seven cases with anti–P-003 reactivity [8]. The frequencies of these two antibodies are the reverse of our observation. This may be because of different frequencies of the DP antigen in the German population, or it may be because the single-antigen bead panel used in the earlier study was not as comprehensive as the current panels and the assignment of some epitopes could have been masked. This observation raises the important question of panel composition. The single antigens represented in the panel should be selected based on the following criteria: 1) the most common alleles should be represented; 2) enough antigens of each serologic group must be selected to allow conclusive inferences to be drawn; and 3) to evaluate the possibility of anti-DPA1 reactivity, DPA1 alleles must be represented in combination with DPB1 alleles of different serologic groups to the extent that it is possible, even if they are not commonly encoded on the same haplotype.

There is substantial evidence for HLA-DQ that indicates that DQA and DQB subunits encoded in cis and trans can be assembled

and form multiple allotypes [9]. There are some restrictions, however, and DQA1 alleles pair with some and not other DQB1 alleles. The common alleles DPA1*0103 and DPA1*0201 present only a few structural differences; they differ by only three amino acid replacements in the alpha-1 domain; these alleles present four additional differences in the remaining segments of the DPA molecule. On the other hand, Begovich *et al.* [10] demonstrated that some alleles of DPB1 may associate with different alleles of DPA1 in different populations. Therefore, given the structural similarities between alleles of DPA1 and the variable DPA1-DPB1 associations, it can be predicted that there are no significant constraints that would prevent trans-complementation in HLA-DP, and up to four different DP moieties in individuals heterozygous in DPA1 and DPB1 could occur. It can be postulated that combinatorial epitopes defined by variations in both DPA and DPB could therefore exist, and these examples should be represented in the single antigen panels. Pairing restrictions, such as those between DQA1 and DQB1, do not appear to be in effect in the case of DP heterodimers. If there were restriction in paring between DPA1 and DPB1, then the restricted alleles would not appear in the same haplotype; selective pressure would prevent them from being in linkage disequilibrium, because homozygous individuals with such restricted DP blocks would not have functional DP molecules. Linkage disequilibrium between heterodimer genes is evidence of the functionality of the corresponding heterodimer molecules. Bogovich *et al.* show that the same DPB1 allele is in linkage disequilibrium with multiple DPA1 molecules.

Because of lower levels of expression of DP molecules on the cell surface as compared with other HLA molecules, anti-DP antibodies do not play such a critical definitive role as anti-A, anti-B, or anti-DR. There is, however, a conclusive correlation between the identification of anti-DP antibodies and crossmatch reactivity; and, to the extent that crossmatch reactivity is used in clinical decisions, a definitive characterization of DP epitopes would be considered a contribution in the practice of clinical transplantation.

In our institution, bone marrow transplantation with unrelated donors is performed with protocols requiring immunosuppression with ATG. The incidence of graft failure is less than 2% in the absence of antibodies. We have observed two isolated cases in which the only apparent explanation of lack of engraftment in hematopoietic cell transplantation was the presence of anti-DP antibodies reacting with epitopes present in the donor. We have also observed how in one hematopoietic cell transplantation case anti-DP reactivity against donor epitopes did not preclude successful engraftment. These observations are not conclusive, and clinical outcome studies of the role of anti-DP antibodies in both solid organ and bone marrow transplantation are still due.

As for the role of T cells and minor histocompatibility antigens in graft rejection in hematopoietic stem cell transplantation, we recently performed a study (pending paper available upon request) of the possible role of anti-HLA antibodies. In this study, donor-specific anti-HLA antibodies were present in 19 of 20 cases of graft rejection. Only 1 case could not be accounted for in terms of an antigen–antibody reaction. In the presence of an antigen–antibody incompatibility in graft failure, a serologic reaction, rather than a T-cell response against minor histocompatibility antigens, is probably the most reasonable explanation. In addition, in the second case presented here, it was precisely the removal of anti-DP antibodies by plasmapheresis resulting in the disappearance of detectable antibodies, the only difference between graft rejection in the first transplantation attempt and engraftment in the second transplantation attempt.

In our daily clinical routine, we do not have the analytical tools to evaluate the role of minor histocompatibility antigens or T-cell response. We operate under the assumption that, in the presence of an antibody against an antigen in the donor, graft rejection is primarily accounted for by a humoral reaction rather than a cellular reaction. This assumption is based on the observations that 1) in almost all cases of graft rejection there was antibody–antigen incompatibility; and 2) in innumerable cases of incomplete HLA matching and absence of antibodies against antigens in the donor, there was not graft rejection.

In addition to what has been stated so far, in evaluating the presence of anti-DP reactivity in serum samples, the following facts must be taken into account. First, compared with other HLA antigens, DP molecules are expressed at a lower level; and second, the effect of anti-DP reactivity on the total intensity of reactivity with a particular cell in a cell panel may therefore be small or even negligible. The advantage of using single-antigen panels for the characterization of HLA specificities of some loci is shown in this study. In the past, when only cell panels were tested, the serologic detection of the DP polymorphism was not accomplished, as the characterization of DP-alloepitopes is extremely difficult in cell panels coexpressing multiple alleles and products of different loci. Third, the contribution of anti-DP reactivity in a crossmatch test may be very small and could be overlooked. Fourth, although the effect of anti-DP antibodies may be small because of the low expression of DP molecules, this effect could be additive to the effect of other antibodies with other specificities. It is the overall reactivity with a particular cell that can result in detectable levels of donor-specific antibodies. Fifth, in predicting the effect of anti-DP antibodies against donor cells, it is essential to rely on high resolution HLA typing of the donor. Sixth, insofar as DPB1 matching is not a priority and is often not even considered in bone marrow transplantation, DPB1 mismatches are very common, as high as 85% in allogeneic transplants with unrelated donors matching in 10 of 10 alleles [11]. Therefore, the presence of anti-DP antibodies in bone marrow transplant candidates becomes particularly important in clinical practice for allogeneic transplantation of organs and hematopoietic stem cells. In all cases, the main practical question is not whether there are anti-DP antibodies but, rather, the intensity of the reactivity displayed by these antibodies. There is a need to establish a standard measure of antibody reactivity on which clinical decisions can be based.

Given the clinical importance of anti-DP antibodies, this study provides the tools to ascertain the presence and identity of anti-DP antibodies, defined in terms of epitopes that have been fully characterized at the molecular level. The role played by the polymorphism of DPA1 in the intensity and specificity of antigen–antibody reactivity with the DP heterodimer remains to be evaluated in more detail. Apart from the four epitopes described here, this study also shows that other possible serologic specificities are a small fraction of the serologic reactivity observed in clinical practice.

In regard to the clinical importance of anti-DP antibodies, we must bear in mind that the relatively lower level of expression of DP molecules on the cell surface has been known from the beginning when DP molecules were still called "SB" [12,13]; and that, because of this lower level of expression, the clinical effect of antigen–antibody reactivity is not as striking and prominent as in the case of antibodies specific for antigens in other loci.

References

[1] Vaidya S, Hilson BM, Sheldon S, Cano P, Fernández-Viña M. DP reactive antibody in a zero mismatch renal transplant pair. Hum Immunol 2007;68:947–9.

[2] Bodmer J, Bodmer W, Heyes J, So A, Tonks S, Trowsdale J, et al. Identification of HLA-DP polymorphism with DP alpha and DP beta probes and monoclonal antibodies: Correlation with primed lymphocyte typing. Proc Natl Acad Sci USA 1987;84:4596–600.

[3] Klohe E, Pistillo MP, Ferrara GB, Goeken NE, Graezel NS, Karr RW. Critical role of HLA-DRB1 residue 58 in multiple polymorphic epitopes recognized by xonogenic and allogenic antibodies. Hum Immunol 1992;35:18–28.

[4] Colonna M, Tanigaki N, Tosi R, Ferrara GB. Serological detection and molecular localization of allelic HLA-DP supertypic epitopes. Eur J Immunol 1989;19: 433–40.

[5] Tanigaki N, Tosi R, Parodi B, Sorrentino R, Ferrara GB, Strominger JL. Detection of HLA-DP serological allodeterminants by the use of radioiodinated DP molecules. Eur J Immunol 1987;17:743–50.
[6] Mazzoleni O, Longo A, Angelini G, Colonna M, Tanigaki N, Delfino L, et al. Human monoclonal antibody MP8 detects a supertypic determinant encoded by DPB alleles DPB2.1, DPB3, DPB4.2, DPB8, DPB9, DPB10, and DPB14. Immunogenetics 1989;30:502–5.
[7] Goral S, Prak EL, Kearns J, Bloom RD, Pierce E, Dolyle A, et al. Preformed donor-directed anti-HLA-DP antibodies may be an impediment to successful kidney transplantation. Nephrol Dial Transplant 2008;23:390–2.
[8] Arnold ML, Pei R, Spriewald B, Wassmuth R. Anti-HLA class II antibodies in kidney transplant patients. Tissue Antigens 2005;65:370–8.
[9] Charron DJ, Lotteau V, Turmel P. Hybrid HLA-DC antigens provide molecular evidence for gene trans-complementation. Nature 1984;312:157–9.
[10] Begovich AB, Moonsamy PV, Mack SJ, Barcellos LF, Steiner LL, Grams S, et al. Genetic variability and linkage disequilibrium within the HLA-DP region: Analysis of 15 different populations [published erratum in Tissue Antigens 2001;58:431]. Tissue Antigens 2001;57:424–39.
[11] Flomenberg N, Baxter-Lowe LA, Confer D, Fernandez-Vina M, Filipovich A, Horowitz M, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. Blood 2004;104:1923–30.
[12] Neppert J, Nunez G, Stastny P. HLA-A, B, C; -DR; -MT, -MB, and SB antigens on unstimulated human endothelial cells. Tissue Antigens 1984;24:40–7.
[13] Nuñez G, Giles RC, Ball EJ, Hurley CK, Capra JD, Stastny P. Expression of HLA-DR, MB, MT and SB antigens on human mononuclear cells: Identification of two phenotypically distinct monocyte populations. J Immunol 1984;133:1300–6.