HLA-DP Alloantibodies

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Can antibodies to mismatched donor HLA-DP antigens cause early rejection and graft loss in renal transplantation? The answer to this question requires identification of DP antibodies, and historically, this is not something that most HLA laboratories have had the tools or inclination to do.

HLA-DP antigens were first identified more than 20 years ago[1] and recognized as primary stimulators of the secondary MLC response. Originally, DP antigens were typed by cellular means, but it wasn't long before antibodies to DP were identified.[2] Antibodies that permitted serological DP typing were investigated in the 10th International Histocompatibility Workshop[3,4] in the late 1980s. This was about the same time that DNA-based HLA typing methods were being developed, and laboratories interested in DP typing dropped serological typing in favor of molecular typing. In the aftermath, DP antibodies, with few exceptions,[5,6] were largely forgotten.

Several years ago, to unravel a renal transplant candidate's crossmatch and screening results, our laboratory at the Puget Sound Blood Center began investigating DP antibodies. Using a class II panel that was typed for DP as well as DR and DQ, we were able to identify a dozen patients who had DP antibodies. Unfortunately, we were only able to identify DP antibodies in the absence of DR and DQ antibodies, as the individual members of the screening panel expressed DR and DQ, as well as DP antigens. Last year, One Lambda Inc. provided our laboratory with an early version of their single antigen class II FlowPRA, beads (see Table 1). Included in the set of beads were six DP antigens, which allowed us to identify DP antibodies regardless of any other HLA antibodies a patient might have.

DR1 (0101)	DR10 (1001)	DR51 (B5*0101)
DR1 (0102)	DR11 (1101)	DR52 (B3*0201)
DR1 (0103)	DR12 (1201)	DR53 (B4*0103)
DR3 (0301)	DR12 (1202)	DQ2 (0201)
DR3 (0302)	DR13 (1301)	DQ4 (0402)
DR4 (0401)	DR13 (1303)	DP1 (0101)
DR4 (0404)	DR14 (1401)	DP2 (0201)
DR4 (0405)	DR15 (1501)	DP3 (0301)
DR7 (0701)	DR15 (1502)	DP4 (0401)
DR8 (0801)	DR16 (1601)	DP5 (0501)
DR9 (0901)		DP11 (1101)

Table 1

Antigens Covered in Single-Antigen Panel

When one considers that there are nearly 100 described DPB1 alleles, the screening of sera for DP antibodies using a panel of six DP antigens might seem inadequate. The polymorphism of DP, like that of the other HLA loci, consists of various combinations of amino acid motifs at a number of polymorphic sites. In the case of HLA-DP, the polymorphism is quite simple. Almost all the different DP antigens can be described as combinations of several different motifs at each of six different sequence positions. This is summarized in Table 2, showing the first 23 DP antigens. In our laboratory's DP typing of more than 200 recipients and donors, we found only 18 different DPB1 alleles (indicated by an asterisk in Table 2). Almost all the polymorphic motifs found in the 18 observed alleles are covered in the six single-antigen DP beads. The only commonly encountered polymorphic motifs not represented in the single-antigen panel are the VH-L motif at position 8-11 (found in 12 percent of our subjects) and isoleucine (I) at position 76 (found in 7 percent of our subjects). Of course, antibodies could also be directed at conformational epitopes that aren't covered by these beads. It's also important to remember that the DP alpha chain is also polymorphic, and alloantibodies can be directed to the DP alpha chain. In fact, the first DP antibody our laboratory ever identified was specific for the alpha chain encoded by DPA1*0103.

We screened our entire renal waiting list (N=738) for class I and class II HLA antibodies using FlowPRA beads (One Lambda). Those patients that were positive for class II antibodies (171 patients) were further tested using the class II single-antigen beads. Table 3 shows the percentage of class II antibody-positive patients having antibodies specifically to DR, DQ, or DP antigens. There were no beads for DQ5, 6, 7, 8, 9, so the actual frequency of DQ antibodies is undoubtedly higher than what is presented here; it probably approaches the frequency of DR antibodies (One Lambda has now released the single-antigen class II beads with DQ5, 6, 7, 8, 9 beads included; in the process the DP beads have been reduced to a single-bead bearing a mixture of DP antigens). DP antibodies are less common than DR and DQ antibodies, but are still found in 12 percent of all our patients, and in half of our patients who have antibodies to class II HLA. Most patients who made DP antibodies also made either DR or DQ antibodies, usually both.

Certain DP antibody specificities were seen repeatedly in our patients. Table 4 shows the most commonly encountered specificities. These specificities can be correlated to particular polymorphic motifs. For example, anti-DP01, 03, 05, 11

DPB1	8-11	33-36	55-57	65-69	76	84-87
0101*	VY-G	E-YA	AAE	I--K	V	DEAV
0201*	LF-G	E-FV	DEE	I--E	M	GGPM
0301*	VY-L	E-FV	DED	L--K	V	DEAV
0401*	LF-G	E-FA	AAE	I--K	M	GGPM
0501*	LF-G	E-LV	EAE	I--K	M	DEAV
1101*	VY-L	Q-YA	AAE	L--R	M	DEAV
0202	LF-G	E-LV	EAE	I--E	M	GGPM
0402*	LF-G	E-FV	DEE	I--K	M	GGPM
0601*	VY-L	E-FV	DED	L--E	M	DEAV
0801	LF-G	E-FV	DEE	I--E	V	DEAV
0901*	VH-L	E-FV	DED	I--E	V	DEAV
1001*	VH-L	E-FV	DEE	I--E	V	DEAV
1301*	VY-L	E-YA	AAE	I--E	I	DEAV
1401*	VH-L	E-FV	DED	L--K	V	DEAV
1501*	VY-G	Q-YA	AAE	L--R	M	VGPM
1601*	LF-G	E-FV	DEE	I--E	M	DEAV
1701*	VH-L	E-FV	DED	I--E	M	DEAV
1801	VY-G	E-FV	DEE	I--K	M	VGPM
1901*	LF-G	E-FV	EAE	I--E	I	DEAV
2001*	VY-L	E-FV	DED	L--K	M	DEAV
2101	VY-L	E-LV	EAE	I--K	M	DEAV
2201	LF-G	E-LV	EAE	I--E	M	DEAV
2301*	LF-G	E-FV	AAE	I--K	M	GGPM

Table 2

Major Polymorphic Sites of HLA-DP

correlates to the DEAV motif at position 84-87, while anti-DP02, 04 correlates to the GGPM motif at the same position. The observed specificities could probably be broadened to include all the DP antigens that bear these motifs. For example, the sera reactive with DP01, 03, 05, 11 are also expected to react with other antigens (DP06, 08, 09, 10, 13, 14, etc.) having the DEAV motif at position 84-87. The sera that appear to be monospecific with the six-antigen panel are also probably motif-specific and are expected to react with other DP antigens having those motifs. For example, the anti-DP03 sera are expected to react with other DP antigens (DP06, 09, 14, etc.) having the DED motif at position 55-57. A few sera had patterns of reactivity that appeared to be directed toward DP alpha.

As with any type of HLA antibody, failed grafts, pregnancies, and transfusions are the primary events that induce DP antibodies. In Table 5, our patients are broken down into three groups that correspond to these sensitization methods. The patients most likely to have developed DP antibodies are those who have lost a previous graft, with 45 percent of our retransplant candidates having DP antibodies. Of the never-transplanted patients, the percentage of female and male patients with DP antibodies (13 percent and <1 percent respectively) shows the relative effects of pregnancy versus transfusion in inducing DP antibodies. We don't have comprehensive pregnancy or transfusion information on all our patients, but a reasonable interpretation of the

89% had DR antibodies
50% had DQ antibodies (DQ5,6,7,8,9 not tested)
52% had DP antibodies

Table 3

Patients with Antibodies to Class II (N=171)

substantially different percentages of male and female patients having DP antibodies is that pregnancy is much more likely than transfusion to induce DP antibodies.

# Patients	Specificity	Probable Target
29	Anti-DP 01, 03, 05, 11	DEAV at position 84-87
18	Anti-DP 03	DED at position 55-57
13	Anti-DP 01	VY-G 8-11 or E-YA 33-36
7	Anti-DP 02, 03	E-FV at position 33-36
6	Anti-DP 03, 11	VY-L at position 8-11
4	Anti-DP 02, 04	GGPM at position 84-87

Table 4

Most Commonly Observed DP Antibody Specificities

Are DP antibodies relevant to transplant success? DP antigens are constitutively expressed on renal microvascular endothelial cells.[7] It is therefore not unreasonable that DP antibodies could cause problems in a transplanted kidney. However, determining whether pre-existing anti-donor DP antibodies are detrimental to a transplanted kidney has been difficult because of the lack of informative testing. The literature regarding this issue is very sparse. Cook[8] described a case of a retransplant from a donor who was mismatched for zero HLA-A, B, DR, DQ antigens with the recipient, but who had a positive B-cell crossmatch. The graft never functioned and was lost at three months. It was concluded that the failure probably was the result of antibodies to a DP epitope shared between the current donor and a previous donor.

Transplant Candidates	Anti-DR	Anti-DQ*	Anti-DP
Previously Transplanted (n=111)	65%	50%	45%
Non-transplanted Females (n=278)	27%	10%	13%
Non-transplanted Males (n=349)	1%	<1%	<1%
All Candidates (N=738)	21%	12%	12%

*DQ5,6,7,8,9 not tested

Table 5

Incidence and Origin of Antibodies to Class II HLA

In our laboratory, we have retrospectively identified two cases where retransplants occurred across positive B-cell crossmatches due to donor-specific DP antibodies (Table 6). Normally, a positive B-cell crossmatch is considered a contraindication to transplant in our laboratory. However, the decision to transplant across a positive B-cell crossmatch in

these cases was made because the recipient and donor were well matched (the DP antibody status of the recipients was unknown at the time). One graft was lost at one month, the other at 11 months. In one recipient, the only class II antibodies present were anti-DP. In the other recipient, the only HLA antibodies present were anti-DP.

Patient #1	Retransplant, 1998:	Zero-DR,DQ mismatch
	Flow crossmatches:	T negative, B weak positive
	Recipient's type:	DPB1*0401, 0402
	First donor's type:	DPB1*0201, 0301
	Second donor's type:	DPB1*0301, 1401
	Pre-txplant antibody:	Anti-DP01,03,05,11 (DEAV)
	Retransplant outcome:	Failed at 1 month
Patient #2	Retransplant, 2000:	Zero-A,B,DR,DQ mismatch
	Flow crossmatches:	T negative, B positive
	Recipient's type:	DPB1*0401, 0402
	First donor's type:	DPB1*0402, 1301
	Second donor's type:	DPB1*0101, 1001
	Pre-txplant antibody:	Anti-DP01,03,05,11 (DEAV)
	Retransplant outcome:	Failed at 11 months

Table 6

Transplants Across a Donor-specific DP Antibody

These cases, though anecdotal, suggest that anti-donor DP antibodies can adversely affect renal allografts. Data from the Collaborative Transplant Study[9] show that matching for DP antigens makes no difference in survival of primary renal allografts, but makes a significant difference in retransplants, especially retransplant patients with elevated PRAs. One possible interpretation of these results is that DP matching itself may have no direct impact on graft survival, but donor-specific DP antibodies can adversely impact graft survival, an effect that is most noticeable in retransplants because these are the recipients most likely to have DP antibodies.

To increase organ sharing, especially for high PRA patients, UNOS is evaluating the idea of "electronic crossmatches," shipping organs based on determinations of acceptable mismatched antigens for a patient. Two approaches for determining acceptable antigens are programs that calculate acceptable mismatches based on the patient's own type (such as HLAMatchmaker) and screening with single-antigen panels. Both of these approaches, unless they take into consideration HLA-DP, are likely to encounter unexpected positive crossmatches for some patients. In our laboratory we are working toward abandoning preliminary crossmatches with deceased donors, relying instead on unacceptable antigen determinations to rule out potential recipients. The first step, identification of antibodies to HLA-A, B, C, DR, DQ, and DP in our transplant candidates, is almost finished. The second step is to add HLA-DP typing to the A, B, C, DR, DQ typing we currently perform on deceased donors.

Most of our transplant candidates who have antibodies to class II HLA also have class II PRAs greater than 80 percent. When testing for class II antibodies with panels expressing DR, DQ, and DP antigens, the antibody specificities of high PRA patients are essentially indecipherable. The use of single-antigen screening panels helps immensely in identifying the specificities of these antibodies, and allows us to see what had hitherto been hidden, that is the rather surprising abundance of antibodies to HLA-DP. The resolving power of single-antigen panels for HLA antibody screening should allow a more definitive determination of the significance of donor-specific DP antibodies in transplantation.

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