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HLA-DP antibodies before and after renal transplantation

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Abstract

Human leukocyte antigen (HLA)-DP is considered a target for humoral immune response in clinical transplantation. This study analyses the incidence of HLA-DP antibodies in renal patients. Development and epitope specificity of donor-specific antibodies (DSA) and non-DSA (NDSA) were examined. Pre- and posttransplant sera of 338 patients were screened for HLA-DP antibodies using the luminex single antigen assay. Positive patients, partners and/or kidney donors were HLA-DP typed by sequence-specific oligonucleotides. Potential epitopes were mapped by comparing the amino acid sequences of HLA-DP hypervariable regions (HVR) A–F of recipient, partner and/or donor. Specificities in the sera were aligned to deduce the HVR motif responsible for the antibodies. HLA-DP antibodies were detected in 14% of the patients (48/338). Before transplantation, the antibodies were shown in 23% (10 females and 1 male) and 77% were found after transplantation (30 in patients after the first, 7 after the second graft). Specificities were never restricted to individual mismatched antigens; broad HLA-DP sensitization was found as a rule. A single HVR mismatch was present in 80% of the DSA and in 79% of the NDSA. No HLA-DPA specific antibodies were found. Our findings confirm that HLA-DP antibodies are specific for epitopes shared by different HLA-DP antigens, indicating that only a restricted number of mismatched epitopes are recognized by the recipients immune system. Matching for immunogenic HLA-DP epitopes for renal transplantation seems to be functionally more relevant than classical matching at the allelic level.

Introduction

Human leukocyte antigen (HLA)-DP is an HLA class II locus identified more than 30 years ago as a possible stimulator of the mixed lymphocyte culture response in previously primed cells. It was originally named the SB locus but was renamed DP after the 1984 workshop (1). DPB1 and DPA1 are the MHC genes encoding the DP molecule. HLA-DP antigens were initially typed by cellular methods, the prevalence of antibodies against HLA-DP antigens was shown later (2, 3). At present, 132 HLA-DP alleles are described using DNA-based typing methods, mostly sequence-based typing (SBT) (4, 5). The polymorphism of HLA-DP is largely concentrated in six hypervariable regions (HVRs), A–F, in exon 2 of the DPB1 gene. Most DP antigens are characterized by specific combinations of amino acid sequences (motifs) of these six HVRs. HLA-DPA is also polymorphic with 27 known alleles at present. They are divided in four groups: DPA1*01–*04. The DPA polymorphism is restricted and involves less amino acids sequences than that of DPB.

HLA-DP antigens expressed on peripheral blood cells and also on endothelial cells especially during rejection episodes are supposed to present a target for the humoral immune response in kidney transplantation (6–8). Population analyses showed that HLA-DP mismatches between donor and recipient did not influence the outcome of first kidney transplants, but did have an impact on retransplants (9). Epitope-based matching rather than allele matching should probably be the method of choice (10).

The contribution of HLA class II antibodies, especially HLA-DP, to graft rejection and graft loss has long been poorly understood, because testing for their detection on B-lymphocytes was hardly satisfactory. With the introduction of the solid-phase antibody detection assays, in particular, the luminex single antigen (LSA) assay, where HLA-DP molecules are attached to microspheres, class II DP antibodies are now detected easily and their clinical relevance might be assessed more accurately (11).

This study was designed to analyze the incidence of HLA-DP antibodies in renal patients before and after transplantation. Furthermore, the epitope specificity and the correlation between the development of donor-specific antibodies (DSA) and nondonor-specific antibodies (NDSA) were examined using LSA. HVRs of both patient and donor were determined by sequence-specific oligonucleotides (SSO) to identify potential DSA epitopes.

Materials and methods

Patients and samples

From January 2007 until January 2009, 338 renal patients from the University Hospital of Maastricht were screened for the presence of HLA class II antibodies using the LSA assay. Of them, 133 are currently on the renal transplant waiting list, where 43 have never been transplanted and 90 are awaiting retransplantation. The remaining 205 were tested in the local posttransplant protocol. All patients presenting with HLA-DP antibodies were selected and investigated further. Their pre- and posttransplant sera were examined with LSA. Patient characteristics are given in Table 1.

HLA-DP antibody detection by LSA assay

Antibodies against HLA-DP in recipient sera were determined using the LABScreen® SA class II assay (One Lambda, Canoga Park, CA) according to the manufacturer's instructions. Microbeads coated with purified HLA molecules were incubated with patient serum for 30 min. After washing to remove unbound antibody, the beads were incubated for 30 min with antihuman-IgG-conjugated phyco-erythrine. All incubations were performed on a gently rotating platform in the dark at room temperature. The LSA class II lot used contained 13 microbeads coated with single HLA-DP alleles: DPB1*0101, *0201, *0301, *0401, *0402, *0501, *0901, *1001, *1101, *1301, *1401, *1701 and *1901. The LABScan 100 flow analyser (Luminex, Austin, TX) and HLA-VISUAL software (One Lambda) were used for data acquisition and analysis. All beads with normalized median fluorescence intensity value (i.e. raw median fluorescence intensity (MFI) value of the test bead − MFI value of the negative control bead) >2000 were considered positive. The cutoff point was established based on the results from previous studies (12).

HLA-DP typing by luminex SSO assay

DNA samples of patients with HLA-DP antibodies ($n = 48$), partners ($n = 8$) and kidney donors ($n = 31$) were typed for HLA-DP using LABType®SSO according to the manufacturer's instructions. For two patients, no DNA from the partner was available and for six recipients DNA of the organ donor was lacking. DNA samples were isolated from either peripheral blood or spleen cells using the QIAamp DNA

Table 1 Characteristics of HLA-DP antibody positive and negative patients: all ($n = 338$), untransplanted ($n = 43$) and transplanted ($n = 295$) patients

All patients	HLA-DP antibody negative patients ($n = 290$)	HLA-DP antibody positive patients ($n = 48$)
Male	145 (50%)	19 (40%)
Females (pregnancies)	145 (121, 83%)	29 (22, 76%)
Number of transplants		
0	32	11
1	180	30
2	67	7
3	6	
4	3	
5	2	
Number of transfusions (mean ± SD)		
Packed cells	4.3 ± 10.0	2.0 ± 2.5
Leukocyte depleted	1.7 ± 3.2	8.6 ± 12.2
Untransplanted	HLA-DP antibody negative patients ($n = 32$)	HLA-DP antibody positive patients ($n = 11$)
Male	8 (25%)	1 (9%)
Females (pregnancies)	24 (24, 100%)	10 (10, 100%)
Number of transfusions (mean ± SD)		
Packed cells	0.7 ± 1.0	2.1 ± 3.5
Leukocyte depleted	1.4 ± 2.9	12.3 ± 21.5
Transplanted	HLA-DP antibody negative patients ($n = 258$)	HLA-DP antibody positive ($n = 37$) patients
Male	137 (53%)	18 (49%)
Females (pregnancies)	121 (97, 80%)	19 (12, 63%)
Number of transfusions (mean ± SD)		
Packed cells	1.8 ± 3.3	2.0 ± 2.2
Leukocyte depleted	4.6 ± 10.5	7.6 ± 7.7

HLA, human leukocyte antigen.

blood mini kit (Qiagen, Venlo, The Netherlands) and stored at −30°C.

In short, 2 µl of purified genomic DNA (concentration 20 ng/µl) is polymerase chain reaction (PCR) amplified using 4 µl of group-specific primer, 0.2 µl Taq polymerase (0.33 U) and 13.8 µl amplification mix. The primer has a biotin label, which allows the PCR product to be detected using *R*-phycoerythrin-conjugated streptavidin (SAPE). After amplification, the PCR product is denaturated and neutralized and allowed to rehybridize (15 min, 60°C) to a mixture of 43 complementary sequence-specific oligonucleotide probes conjugated to color-coded beads. The mixture is then washed three times and is incubated with 50 µl of SAPE (1:50) for 5 min at 60°C. Excess SAPE is washed away. The LABScan 100 flow analyser (Luminex) identifies the fluorescent intensity of the PE on each microsphere. The assessment of the HLA-DP typing is based on the reaction pattern compared with patterns associated with published HLA-DP sequences.

Twelve DNA samples previously typed for HLA-DP by the SBT method described previously (13) were used to validate the SSO HLA-DP typing method, which represented the alleles DPB1*0101, *0201, *0301, *0401, *0402, *0501, *0901, *1001, *1101, *1301, *1401, *1701 and *1901. The aim was to correctly identify the different polymorphic motifs of the six HVRs of exon 2 of all HLA-DP alleles present in the LSA class II lot used (Table 2). The results obtained were identical to SBT, albeit SSO included ambiguous typing results. Among those, the correct typing was always present, for all samples the six HVR polymorphisms were correctly identified. In case an ambiguous result was obtained for patient and partner/donor HLA-DP typing, the allele most frequent in Caucasians was assigned (14).

Donor specificity of HLA-DP antibodies

Epitopes for possible antibody formation were mapped by comparing the HLA-DP HVR amino acid sequences of exon 2 of recipient, partner and/or donor, as defined by SSO typing. A potential mismatched epitope was defined as an amino acid sequence present in one of the six HVRs of partner or donor, but absent in the recipient. To verify the potential epitopes, LSA patterns were used (Table 2). The amino acid sequences of the HLA-DP-specificities recognized by the serum were aligned. They were compared in order to deduce the most likely mismatched HVR motif responsible for the antibodies. An example is given in Table 3.

Results

HLA-DP antibody incidence in renal patients

Screening for HLA class II antibodies by LSA before and after transplantation was performed for 338 renal patients from the University Hospital of Maastricht. Class II HLA-DP antibodies were detected in 48 (14%) of them. In 11 patients (23%), DP antibodies were detected before transplantation, 10 females and 1 male. In 37 of the positive patients (77%), the antibodies were shown after transplantation, in 30 after the first and in 7 after the second graft (Table 1). In two patients, DP antibodies were the only ones demonstrable; HLA-DR and -DQ antibodies were present next to DP in 41 (Table 4). Additional class I antibodies were shown in 45 patients.

HLA-DP typing by luminex SSO assay

The amino acid sequences of the six HVRs of exon 2 of all HLA-DP alleles present in the LSA class II lot used (Table 2) were correctly identified by SSO in all samples. An ambiguous result in patient and partner/donor HLA-DP typing was obtained in three individuals (ID7 DPB1*0601, ID10 DPB1*1601 and ID11 DPB1*3601) (Table 6).

Table 2 Hypervariable regions (A–F) of exon 2 of the HLA-DPB1 alleles present in the LSA class II assay

DPB1*	A 8–11	B 33–36	C 55–57	D 65–69	E 76	F 84–87
*0101	VYQG	EEYA	AAE	ILEEK	V	DEAV
*0201	LFQG	EEFV	DEE	ILEEE	M	GGPM
*0301	VYQL	EEFV	DED	LLEEK	V	DEAV
*0401	LFQG	EEFA	AAE	ILEEK	M	GGPM
*0402	LFQG	EEFV	DEE	ILEEK	M	GGPM
*0501	LFQG	EELV	EAE	ILEEK	M	DEAV
*0901	VHQL	EEFV	DED	ILEEE	M	DEAV
*1001	VHQL	EEFV	DEE	ILEEE	V	DEAV
*1101	VYQL	QEYA	AAE	LLEER	M	DEAV
*1301	VYQL	EEYA	AAE	ILEEE	I	DEAV
*1401	VHQL	EEFV	DED	LLEEK	V	DEAV
*1701	VHQL	EEFV	DED	ILEEE	M	DEAV
*1901	LFQG	EEFV	EAE	ILEEE	I	DEAV

HLA, human leukocyte antigen; LSA, luminex single antigen.

Table 3 Comparison of patient and donor hypervariable region (HVR) typing (SSO) to identify potential epitopes (bold) and alignment of specificities detected with LSA to verify donor-specific epitope (boxed)

		HVR					
SSO	HLA-DP	A	B	C	D	E	F
Patient 18876	*0201	LFQG	EEFV	DEE	ILEEE	M	GGPM
	*0401	LFQG	EEFA	AAE	ILEEK	M	GGPM
Donor	*0101	**VYQG**	**EEYA**	AAE	ILEEK	**V**	**DEAV**
	*0401	LFQG	EEFA	AAE	ILEEK	M	GGPM
		HVR					
LSA	DP	A	B	C	D	E	F
Positive beads	*1901	LFQG	EEFV	EAE	ILEEE	I	**DEAV**
	*0101	**VYQG**	**EEYA**	AAE	ILEEK	**V**	**DEAV**
	*1701	VHQL	EEFV	DED	ILEEE	M	**DEAV**
	*0301	VYQL	EEFV	DED	LLEEK	**V**	**DEAV**
	*0501	LFQG	EELV	EAE	ILEEK	M	**DEAV**
	*1001	VHQL	EEFV	DEE	ILEEE	**V**	**DEAV**
	*1301	VYQL	**EEYA**	AAE	ILEEE	I	**DEAV**
	*1401	VHQL	EEFV	DED	LLEEK	**V**	**DEAV**
	*0901	VHQL	EEFV	DED	ILEEE	M	**DEAV**
	*1101	VYQL	QEYA	AAE	LLEER	M	**DEAV**
Negative beads	*0402	LFQG	EEFV	DEE	ILEEK	M	GGPM
	*0201	LFQG	EEFV	DEE	ILEEE	M	GGPM
	*0401	LFQG	EEFA	AAE	ILEEK	M	GGPM

HLA, human leukocyte antigen; LSA, luminex single antigen; SSO, sequence-specific oligonucleotide.
Bold: the potential mismatched epitope, defined as an amino acid sequence present in one of the six HVRs of the partner or donor, but absent in the recipient.
Boxed: the mismatched DEAV motif considered to be responsible for the HLA-DP antibodies detected by LSA.

Table 4 Class II antibodies detected by LSA in HLA-DP positive and negative patients

	DP antibody negative patients ($n = 290$)	DP antibody positive patients ($n = 48$)
DR + DQ	74 (25.5%)	27 (56%)
DR only	48 (16.5%)	10 (21%)
DQ only	25 (9%)	4 (8%)
DR–DQ negative	143 (49%)	7 (15%)

HLA, human leukocyte antigen; LSA, luminex single antigen.

Pretransplant HLA-DP antibodies

All female patients positive for HLA-DP antibodies before transplantation had been pregnant. For two patients, the immunization partner was unknown and no children were available for typing. For the remaining eight patients, DNA of the partner was available for retrospective DP typing to identify the potential epitopes to which the antibodies were directed. Two patients proved to be DP identical with their partner, the remaining six were mismatched for one or two DP alleles. Patient DP typing, allelic and HVR mismatches are given in Table 5. In 5/6 DP-mismatched patients, DSA were detected. Additional NDSA specificities directed against a single donor-specific HVR mismatch were detected in three patients and against two donor-specific HVR mismatch in one. In one patient with four HVR mismatch, no clear motif could be detected. One patient had detectable NDSA only; her DP antibodies were the result of interlocus DRB1/DPB1 cross-reactivity. The same held true for the DP antibodies of the two patients, who were DP identical to their partner.

In the two females with unknown pregnancy immunization, two antibodies-causing motifs were identified. The only male patient, who presented with pretransplant DP antibodies, had received 66 transfusions with leukocyte-depleted blood. In his serum, class I antibodies were present but no additional class II DR/DQ antibodies were detected. No identification was possible for the antibody-causing motif.

Posttransplant HLA-DP antibodies

HLA-DP antibodies after transplantation were found in 37 recipients: in 30 after their first transplant and in 7 after the second. For 31 of them, donor-DNA was available for retrospective HLA-DP typing. Sixteen recipients were mismatched for two DP alleles with their donor, fifteen showed one allelic DP mismatch. Patient DP typing, allelic and HVR mismatches are given in Table 6.

DSA were found in 26 recipients, NDSA were shown in all. DSA and NDSA were directed against a single donor-specific HVR in 19 patients, against 2 HVRs in 2 and against 3 HVRs

Table 5 HLA-DP antibodies in 11 patients before transplantation

Patient HLA-DP			Pregnancies			Anti-DP antibodies		
ID			Number	Allelic DP mm	DP HVR mm	DSA	NDSA	Epitopes causing DP antibodies
1	0501	1601	2	0201 0402	F	**0201 0402**	0401	**F (GGPM)**
2	0101	0401	4	0201 1701	A B C D	**0201 1701**	0301 0402 0901 1001 1401	**C (DED/DEE)**
3	0401	0402	2	0101	A B E F	**0101**	0401 1001 1301	NIP
4	0401		4	0301	A B C D E F	**0301**	0101 0201 0402 0501 0901 1001 1101 1301 1401 1701 1901	**B (EEFV)**, + C (DEE), **F (DEAV)**
5	0301	0401	6	1301	B D E		0201 0402 1001	C (DEE)
6	0402	2801	2	1401	A C D E F	**1401**	0101 0301 0501 0901 1001 1101 1301 1701 1901	**F (DEAV)**
7	0501	1301	1	zero mm			0201 0301 0402 0901 1001 1401 1701	C (DED/DEE)
8	0401		2	zero mm			0201 0301 0402 0901 1001 1401 1701	C (DED/DEE)
9	0201	0401	2	UNK			0101 0301 0501 0901 1001 1101 1301 1401 1701 1901	C (AAE/DED/DEE), F (DEAV)
10	0201	0402	5	UNK			0101 0301 0501 0901 1001 1101 1301 1401 1701 1901	C (AAE/DED/DEE), F (DEAV)
11	0401						0101 0201 0301 0501 0901 1001 1301 1401 1701 1901	NIP

HLA, human leukocyte antigen; HVR, hypervariable region; NIP, no identification possible; mm, mismatch; UNK, unknown.
The only male patient (ID11) received 66 U of leukocyte-depleted blood. Bold signifies donor-specific antibodies.

in 1. The antibody-causing motif was donor-specific in 25, and nondonor-specific in 4 recipients. For eight recipients, no clear motif could be identified.

Frequency of antibody-target motifs

A total of 47 antibody-target patterns were observed. The HVR-F DEAV motif was detected in 23 (49%) cases, HVR-C (DED/DEE) in 14 (30%), HVR-B (EEFV) in 6 (13%) and HVR-C (AAE), HVR-F (GGPM), HVR-D (ILEEE) and HVR-E (V) in 1 case each. In 70% ($n = 33$), the antibody-target motif was donor specific.

Discussion

Anti-HLA-DP antibodies have always been difficult to identify by complement dependent cytotoxicity (CDC). In most instances, CDC panels were not routinely typed for HLA-DP, and if typing was performed, the specificity of the antibodies was difficult to establish because they were not directed toward a single DP antigen. Furthermore, DP antibodies were often masked by the presence of other class II antibodies. Recently, microbeads coated with purified HLA-DP molecules were added to the SA class II assay, both in flow cytometry and luminex, which allows more precise identification of HLA-DP antibodies. In the present study, renal patients were screened for HLA-DP antibodies using a panel of 13 HLA-DP antigens: *0101, *0201, *0301, *0401, *0402, *0501, *0901, *1001, *1101, *1301, *1401, *1701 and *1901. Although over 130 HLA-DPB1 alleles have been described at present, the most frequent alleles in the Caucasian population are represented in this panel (14). Because the polymorphism of HLA-DP is concentrated in six HVRs of exon 2 of the HLA-DPB1 gene and almost all DP antigens can be described as combinations of several different motifs at each of these HVRs (Table 2), the panel used covered all the important and most frequent HVRs present in the Caucasian population. The polymorphic HVR motifs of exon 2 of HLA-DP not represented in the panel were: VDQL at position 8–11 (DPB1*7001), DEV (*3201) and EEE (*8401) at position 55–57, LLEEE (*0601, 2901, 4401, 9501), FLEEE (*4101), NLEEK (*6001) and FLEEK (*8301) at position 65–69 and VGPM (*1501, *1801, 2801, 3401, 4001, 5301, 6201, 7401) and NEAV (*2202) at position 84–87. The LLEEE motif was present in two donors typed as DPB1*0601 (Table 6, ID7 and ID11). The VGPM motif was present in one patient typed as DPB1*2801 (ID6, Table 5).

The SSO assay for HLA-DP typing was validated for the alleles listed in Table 1. In 30% of the validation samples, the SSO result was the same as in SBT. In 70%, SSO resulted in ambiguous results, always including the correct SBT-DP typing. These results were because of polymorphisms outside the six HVRs, for which no DNA probes were available in the SSO assay. Nevertheless, all polymorphic motifs of the six HVRs of exon 2 could be correctly assigned using the SSO

Table 6 HLA-DP antibodies in 30 patients after the first (ID1–30) and in seven patients after the second transplantation (ID31–37)

		Tx				Anti-DP antibodies		
ID		Number	Allelic DP mm		DP HVR mm	DSA	NDSA	Epitopes causing DP antibodies
1	0402	1	0101	0201	A B C D E F	**0101**	0301 0501 0901 1001 1101 1301 1401 1701 1901	**F (DEAV)**
2	0401 0402	1	0101	0301	A B C D E F	**0101 0301**	0501 0901 1001 1101 1301 1401 1701 1901	**F (DEAV)**
3	0201 0401	1	0101	0501	A B C E F	**0101 0501**	1001 1301 1401 1701 1901	NIP
4	0402	1	0201	0402	B C D		0101 0301 0501 0901 1001 1101 1301 1401 1701 1901	F (DEAV)
5	0201 0202	1	0301	0901	A C D E F	**0301 0901**	0101 1001 1401 1701 1901	**E (V)**
6	0201	1	0401	0501	B C D F	**0501**	1001 1701	NIP
7	0201	1	0401	0601	A B C D F		0101 0301 0501 0901 1001 1101 1301 1401 1701 1901	**F (DEAV)**
8	0201 0401	1	0402	0501	B C F	**0501**	0101 0301 0901 1001 1101 1301 1401 1701 1901	**F (DEAV)**
9	0101 0401	1	0402	0601	B C D	**0402**	0201 0301 0901 1001 1401 1701	**C (DED/DEE)**
10	0401	1	0402	1601	B C D F	**0402**	0201 0301 0901 1001 1401 1701 1901	**B (EEFV)**
11	0401 0402	1	0601	3601	A B C D F		0101 0301 0501 0901 1001 1101 1301 1401 1701 1901	**F (DEAV)**
12	0301 0401	1	1301	1401	A B D E		0301 1701 1901	NIP
13	0301 0402	1	1301	1401	A B C D E	**1301**	0101 0501 1001 1301 1701 1901	NIP
14	0201 0402	1	0101		A B E F	**0101**	0301 0501 0901 1001 1101 1301 1401 1701 1901	**F (DEAV)**
15	0101 1101	1	0201		A B C D F	**0201**	0301 0401 0402 1101 1901	NIP
16	0401	1	0201		B C D	**0201**	0301 0402 0901 1001 1401 1701	**C (DEE)** C (DED)
17	0401 0402	1	0201		D		0101 0301 1101 1701 1901	NIP
18	0401	1	0301		A B C D E F	**0301**	02010901 1001 1401 1701	**C (DED)** C (DEE)
19	0201 0401	1	0301		A C D E F	**0301**	0101 0501 0901 1001 1101 1301 1401 1701 1901	**F (DEAV)**
20	0401 0801	1	0301		A C D	**0301**	0901 1401 1701	**C (DED)**
21	0401 0501	1	0301		A B C D E	**0301**	0201 0402 0901 1001 1401 1701 1901	**B (EEFV)**
22	0401	1	0301		A B C D E F	**0301**	0101 0201 0402 0501 1001 1101 1301 1401 1701 1901	**B (EEFV) F (DEAV)**
23	0201 0401	1	0301		A C D E F	**0301**	0101 0501 0901 1001 1101 1301 1401 1701 1901	**F (DEAV)**
24	0401	1	0402		B C	**0402**	0201 1001	**B (EEFV) C (DEE)**
25	0401	1	1001		A B C D E F	**1001**	0101 0201 0301 0402 0501 0901 1101 1301 1401 1701 1901	**F (DEAV) B (EEFV) C (DEE)**
26	0401 0402	1	1701		A C D F	**1701**	0101 0301 0501 0901 1001 1101 1301 1401 1901	**F (DEAV)**
27	0401	1	UNK				0901 1001 1701	NIP
28	0401 0402	1	UNK				0101 0301 0501 0901 1001 1101 1301 1401 1701 1901	F (DEAV)
29	0401	1	UNK				0101 0201 0301 0501 0901 1001 1101 1301 1401 1701 1901	F (DEAV) D (ILEEE)
30	0401 0501	1	UNK				0201 0301 0402 0901 1001 1401 1701	C (DED/DEE)
31	0401	2	0101	0301	A B D E F	**0101 1301**	0301 0501 0901 1001 1101 1401 1701 1901	**F (DEAV)**
32	0201 0401	2	0101	0301	A B C D E F	**0101 0301**	0501 0901 1001 1101 1301 1401 1701 1901	**F (DEAV)**
33	0201 0402	2	0401	1401	A B C D E F	**1401**	0101 0301 0501 0901 1001 1101 1301 1701 1901	**F (DEAV)**
34	0401	2	0101		A B E F	**0101**	0301 0501 0901 1001 1101 1301 1401 1701 1901	**F (DEAV)**
35	0401	2	1301		A B D E F	**1301**	0101 0301 0501 0901 1001 1101 1401 1701 1901	**F (DEAV)**
36	0101 0402	2	UNK				*0301 0901 1701	C (DED)
37	0401	2	UNK				*0101 0201 0301 0501 0901 1001 1401 1701 1901	NIP

DSA, donor-specific antibodies; HLA, human leukocyte antigen; HVR, hypervariable region; NDSA, non donor-specific antibodies; NIP, no identification possible; UNK, unknown.
Bold signifies donor-specific antibodies.

method. In only three cases, we had to assign an allele that was not validated.

Before the introduction of solid-phase techniques for HLA-DP antibodies detection, DP antibodies were studied using the monoclonal antibody immobilization of leukocyte antigens (MAILA) technique. In 1990, Mueller-Eckhardt described DP antibodies in 9.7% of pregnancy sera using MAILA and showed a high correlation of HLA-DP immunization together with reactivity against HLA class I and II (DR/DQ) antibodies (15). Using the same technique, Pfeiffer in 1995 found the overall incidence of HLA-DP antibodies in patients awaiting renal transplantation to be 7.3% (16). Using flow-cytometric beads, Youngs reported that 12% of patients on their renal transplant waiting list possessed DP antibodies. In previously transplanted patients, the percentage was even higher (45%) (17). With the same technique, Qiu described an increased incidence of DP antibodies after graft rejection (11). In the present study, we found 12% of renal patients to have DP antibodies, 23% before and 77% after transplantation.

Although the HLA-DPA chain is also polymorphic and could potentially be the source of antibody reactivity, no HLA-DPA specific antibodies were found in this study. In a population of this size, one might expect to find DP antibodies reacting with DPA. The LSA class II kit used contained four beads carrying DPA*0103, eight beads coated with DPA1*0201 and one bead with DPA1*0401. To evaluate possible anti-DPA reactivity, DPA1 alleles have to be represented in different combinations with DPB1 alleles, even if those are not commonly encoded on the same haplotype. DPA antibodies were suspected in sera where the DPB specificities remained undetermined, especially in three patients whose serum reacted with their own DPB1 allele (Table 5 ID3, Table 6, ID12 and ID15); however, this was not confirmed by single antigen analysis. The role of the polymorphism of DPA1 in the intensity and specificity of antigen–antibody reactivity with the DP-heterodimer remains to be investigated further. DPA typing of patients, partners and donors was not performed because no DPA antibodies were shown.

Because of the weak linkage disequilibrium of HLA-DP with the classical HLA class I and II antigens (18), even donor–recipient pairs identical for these loci have an 80% probability of being mismatched at DP (19). However, because of the restricted DP polymorphism, many of these allelic mismatches will be compatible for HVR motifs and, therefore, will not result in anti-DP formation.

For most of the patients, the immunizing HLA-DP antigens and subsequent HVR mismatches could be determined. In 80% the DP antibodies were donor specific. None of the patients developed antibodies restricted to the mismatched DP antigens, and broad DP sensitization was found as a rule. This finding confirms previous observations that DP antibodies in sera of sensitized patients are specific for epitopes shared by different HLA antigens (17, 20, 21). Even though many more potential HVR mismatches were present, almost all the DSA (80%) and accompanying NDSA (79%) were directed against a single donor-specific HVR mismatch: HVR-F in 16, HVR-C in 3, HVR-B in 2 and HVR-E in 1. This indicates that only a small number of mismatched epitopes are recognized by the recipients immune system and supports the immunodominance of HVR-F and -C as also described by Duquesnoy et al. (21). HVR-F and -C correspond to well-defined serological epitopes recognized by monoclonal antibodies (22, 23). In 35 patients homozygous for GGPM (HVR-F) 69% of the DP antibodies were directed against the DEAV motif at that same position. In 21 patients homozygous for AAE/EAE (HVR-C) 48% of the DP antibodies were directed against the DED/DEE motif at that same position. These findings support the findings by Laux et al. (10) that matching for immunogenic DPB1 epitopes was functionally more relevant than classical matching at the allelic level (9, 24).

As first described by Bodmer et al. (22, 25), DR11 has the amino acids D and E at positions 57 and 58 that correlate with the D and E in HVR-C (position 56–57) of 7 of the 13 HLA-DP alleles present in the LSA assay (DPB1*0201, 0301, 0402, 0901, 1001, 1401 and 1701). In five patients with strong anti-DR11 antibodies, the DP antibodies probably resulted from this interlocus DR11-DP epitope. In two patients, who were HLA-DP identical to their partner and had pretransplant DP antibodies, and in one patient with posttransplant DP antibodies, the DP antibodies were directed against the DED and DEE motif of HVR-C. In another patient with pretransplant DP antibodies, the specificities were explained by the DEE motif of HVR-C because the patient possessed the DED motif herself. In another patient, the specificities were explained by the DED motif of HVR-C, while the patient himself carried the DEE motif.

HLA-DP as well as HLA-DQ are upregulated upon inflammation as shown earlier (8). This might be the reason of the clinical relevance of anti-DP antibodies in retransplants (9). The contribution of anti-HLA-DP antibodies to kidney graft rejection and failure has long been an unresolved issue. With the introduction of the SA, the presence of HLA-DP antibodies can be detected easily. Subsequently, it will become possible in the near future to assess their clinical relevance more accurately. Meanwhile, some case reports have recently shown that DP antibodies represent a possible risk factor for transplant dysfunction and failure (25–27).

In summary, we have shown that HLA-DPB antibodies are found in patients before and after kidney transplantation. Before transplantation, pregnancy is the most important stimulus for antibody production but also transfusions may result in DP antibody positivity. The majority of the DP antibodies, however, are found after transplantation, usually already after the first graft. No HLA-DPA-specific antibodies were found in this study. The DP antibodies detected in sera of sensitized patients are specific for a number of epitopes shared by different HLA antigens. The majority of the DSA and accompanying NDSA found are directed against a single donor-specific

HVR mismatch, which indicates that only a small number of mismatched epitopes are recognized by the recipients immune system. Our findings confirm that matching for immunogenic HLA-DP epitopes seems to be functionally more relevant than classical matching at the allelic level, whether this is only true for retransplants remains to be established.

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