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K. Pfeiffer
U. Vögeler
K. H. Albrecht
F. W. Eigler
B. Buchholz
H. Grosse-Wilde

HLA-DP antibodies in patients awaiting renal transplantation

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K. Pfeiffer · U. Vögeler · H. Grosse-Wilde (✉)
Institut für Immunologie,
Universitätsklinikum Essen,
Virchowstraße 171, D-45122 Essen,
Germany
Fax: + 49 201 723 5906

K. H. Albrecht · F. W. Eigler
Abteilung für Allgemeine Chirurgie,
Universitätsklinikum Essen,
Hufelandstr. 55, D-45122 Essen,
Germany

B. Buchholz
Chirurgische Klinik,
Universitätsklinikum Münster,
Germany

Abstract Sera from 505 patients awaiting renal transplantation with known panel-reactive cytotoxic antibody (PRA) status were tested for HLA-DP antibodies of the IgG class by means of the monoclonal antibody immobilization of leukocyte antigens (MAILA) technique. The overall incidence of HLA-DP antibodies was 7.3 %. A positive HLA-DP antibody status correlated only weakly ($r = 0.23$) with a positive cytotoxic antibody status. After retrospective analysis, patients with HLA-DP antibodies prior to retransplantation revealed a significantly ($P < 0.025$) higher graft function rate than HLA-DP-negative patients. One patient was found to possess IgG HLA-DP autoantibodies prior to transplantation; thus far, his graft has been functioning for more than 2 years.

Key words HLA-DP antibodies, kidney transplantation

Introduction

Based on the report of Patel and Terasaki [10], it is established that cytotoxic alloantibodies to HLA class I alloantigens have a deleterious effect on graft function rate (GFR). Therefore, the lymphocytotoxicity test (LCT) to identify sensitized patients is a routine tool before renal transplantation. Using dithiothreitol (DTT), the LCT is able to differentiate between cytotoxic antibodies of the IgM and IgG classes. IgM antibodies have been described to have less harmful effects on the transplant [1, 13]. Very recently, HLA class I antibodies of the IgA type were reported with protective effects on the graft [6]. Attempts to relate the specificity and immunoglobulin classes of HLA antibodies to GFR gave diverging results: HLA class I or class II (HLA-DR)-specific antibodies seemed to be associated with a lower GFR [7, 11]. However, HLA-DQ antibodies were found to have no effect on the graft in one study [12] and deleterious effects in another [4].

We analyzed the incidence and specificity of HLA-DP antibodies of the IgG class in patients awaiting renal transplantation and tried to determine whether HLA-DP antibodies affect the outcome of renal transplantation.

Patients, materials, and methods

Patients

Serum samples of all patients ($n = 505$) on the renal transplantation waiting list in April 1989 at the transplant centers Essen and

Table 1 HLA-DP types of reference LCL used (*IHW No.* international histocompatibility workshop number, *Id No.* local identification, *DPB1** allele designation, *DPw* specificity according to PLT)

IHW No.	Id No.	HLA-DPB1*	DPw
9049	IBW9	0101,0101	1,1
9023	VAVY	0101,0101	1,1
9068	BM9	0201,0201	2,2
9038	BM16	0201,0201	2,2
0607	KRA	0301,0301	3,3
9059	SLE005	0301,0301	3,3
9061	ABO31227	0401,0401	4,4
0807	BAE	0401,0401	4,4
9055	HO301	0501,0501	5,5
9073	KT12	0501,0501	5,5
0701	BOR	0201,0601	2,6
9063	WT47	1601,1601	2,2
9066	TAB089	0202,0202	–,–

Table 2 Distribution of lymphocytotoxic and HLA-DP-specific antibodies in 505 kidney patients [HLA-DP Ab + HLA-DP antibodies present, HLA-DP Ab –: HLA-DP antibodies absent, PRA +: panel reactive antibodies present (> 5 PRA %), PRA –: panel reactive antibodies absent]

	HLA-DP Ab +	HLA-DP Ab –	Total
PRA +	20	83	103 (20.4 %)
PRA –	17	385	402 (79.6 %)
Total	37 (7.3 %)	468 (92.3 %)	505 (100 %)

$\chi^2 = 27.9$; $r = 0.23$; $P < 0.001$

Münster were included in the study. Cytotoxic antibody incidence and specificity to HLA-A and -B antigens of these sera were known from routine screening programs using peripheral blood lymphocytes from HLA-typed healthy donors. The sera were stored at – 20 °C until use. The individual GFR was defined as the period between the day of transplantation and the day on which the patient returned to dialysis due to a nonfunctioning graft.

Lymphoblastoid cell lines (LCL)

As a reference for HLA-DP specificities, 13 LCL defined at the 9th and 10th International Histocompatibility Workshops (IHW) were selected for the study (Table 1). For HLA-DPw1–5, two unrelated LCL each were used, whereas for HLA-DPw6 there was only one heterozygous LCL available. LCL WT47, typed by primed lymphocyte typing (PLT) as HLA-DPw2 at the 10th IHW, is now known as DPB1*1601 homozygous. LCL TAB089, representing an undefined PLT specificity, is typed by DNA analysis as homozygous for DPB1*0202.

Monoclonal antibody immobilization of leukocyte antigens (MAILA)

The MAILA assay employed in the present study uses LCL expressing HLA-DP as the antigen source. Coincidental incubation of LCL with an HLA-DP-specific murine monoclonal antibody (mAb) and the serum of the proband can lead to formation of trimolecular complexes consisting of HLA-DP antigen, mAb, and alloantibody, provided the serum of the proband contains HLA-DP alloantibodies. After lysis of LCL, a microtiter plate coated with antibodies against murine Ig is utilized to capture these complexes via the bound HLA-DP mAb. By adding enzyme-conjugated anti-human IgG antibodies and substrate, HLA-DP-specific alloantibodies become detectable by color reaction.

The MAILA test procedure was performed as described by Mueller-Eckhardt et al. [8]. In brief, the above LCL in culture were harvested, washed once, adjusted with phosphate-buffered saline (PBS) at a concentration of 2.5×10^6/ml incubated v/v with paraformaldehyde (2 %) solution at 4 °C for 5 min. The fixed LCL were washed three times and resuspended in PBS supplemented with 2 % bovine serum albumin and 0.1 % sodium azide (PBS-BSA) at a concentration of 10^7/ml. When needed, cells were stored at 4 °C until use.

Aliquots of 2×10^6 fixed LCL were transferred into Beckman tubes and resuspended by adding 50 µl PBS, 40 µl patient serum, and 40 µl of the HLA-DP-specific mAb BRA Fb6 [3, 5]. After 30 min of incubation at 37 °C, the cells were washed three times with 500 µl isotonic saline and subsequently lysed by adding 100 µl TRIS lysis buffer containing 0.5 % Triton-X100 and treated in an ultrasonic water bath for 5 min. To remove large cell fragments, the suspension was centrifuged for 30 min at 13,000 *g* and 4 °C. Fifty microliters of the supernatant was diluted 1:5 with TRIS buffer and 100 µl aliquots were delivered as duplicates into microtiter plates prepared as follows.

Flat-bottomed microtiter plates (Immuno Plate Maxisorb F96, Nunc, Wiesbaden, Germany) were coated overnight at 4 °C with 100 µl/well of an 1:500 diluted goat anti-mouse IgG(Fc) antiserum (Dianova, Hamburg, Germany), washed three times and blocked by TRIS buffer.

After an incubation period of 90 min at 4 °C, the plates were washed four times with TRIS buffer, followed by the addition of 100 µl of a 1:500 diluted alkaline phosphatase-conjugated goat anti-human IgG antibody (Dianova, Hamburg, Germany) and further incubated for 120 min at 4 °C. The plates were washed five times, 100 µl of substrate solution containing paranitrophenylphosphate was added, and incubated for 30 min at 37 °C in the dark. The color development was stopped by 50 µl 3 N NaOH per well.

The enzyme reactions were read as optical density (OD) values at 405 nm in an EL309 Microplate Autoreader (Biotek Instruments, Winooski, Vt., USA). Extinctions were expressed as mean values of duplicates minus blank extinctions. The serum of a male, nontransfused donor served as a negative control. The positive control was a serum reported to possess HLA-DP-specific alloantibodies (kindly provided by Prof. Dr. G. B. Ferrara, IST Genoa, Italy). To define the cut-off level for the MAILA, OD values obtained from 48 sera without cytotoxic panel-reactive antibodies (PRA) and no history of pregnancy or blood transfusions of the donors were analyzed. The 99.5 percentile reached 0.350 OD. Thus, an OD value equal or greater than 0.350 was considered positive.

Statistical analysis

For comparison of the various groups analyzed, the χ^2-test was used. The corresponding *r*-values were approximated as $r = (\chi^2 : N)^{\frac{1}{2}}$.

Table 3 Characteristics of 37 patients identified with HLA-DP antibodies (*ET-No* Eurotransplant number, *PRA %* current PRA status, *α-HLA* HLA specificity of PRA, *TR* number of blood transfusions, *PR* number of pregnancies, *TX* number of previous grafts, *GFR* graft function rate in days)

	ET-No	Sex	PRA (%)	α-HLA	TR	PR	TX	GFR
01	27273	M	0		2	–	0	
02	29951	F	0		6	–	0	
03	23653	M	0		2	–	0	
04	29099	M	0		8	–	0	
05	33642	F	0		10	–	0	
06	5185	F	0		6	–	0	
07	30332	M	0		1	–	0	
08	28376	M	0		3	–	0	
09	17417	F	0		10	3	0	
10	19222	F	0	B49, 51	2	1	0	
11	19715	M	0		1	–	0	
12	26804	F	6		3	5	0	
13	31431	F	28		2	1	0	
14	8958	M	33		1	–	0	
15	34122	F	38	B44	10	–	0	
16	32428	F	42	A1	15	2	0	
17	5338	F	63	A2, 9, 28	7	–	0	
18	8568	M	63	A2, 9, 28	1	–	0	
19	32217	F	81	B8, Bw6	0	2	0	
20	2445	F	93	A2, B8	60	–	0	
21	10359	F	96	A1, 11	1	3	0	
22	23983	M	0		2	–	1	641
23	27844	F	0		2	2	1	1
24	6110	M	0		7	–	1	536
25	16477	M	0		3	–	1	1
26	11787	F	0		1	–	1	840
27	12048	M	0		1	–	2	8
28	21868	F	11		10	–	1	974
29	3203	F	18	B12, 40	4	3	1	2620
30	9234	M	28	A1, 9, 11	99	–	1	2916
31	19878	M	29	B5, 35	6	–	1	587
32	11232	M	44	A2	20	–	1	409
33	24661	M	53	Bw4	14	–	1	97
34	19935	F	61	A2	38	–	1	486
35	9539	F	69	A1, 3, 11	40	–	1	233
36	22392	M	71	Bw4	15	–	1	2
37	15661	F	79		3	6	2	1

Table 4 HLA-DP antibody status in patients awaiting first transplant (0 TX) or retransplant (≥ 1 TX)

	HLA-DP Ab +	HLA-DP Ab –	Total
0 TX	21	429	450
1 ≥ TX	16	39	55
Total	37	468	505

Results

Of the 505 serum samples tested, 103 (20.2 %) revealed a positive PRA status and 37 (7.3 %) contained HLA-DP-specific IgG antibodies. There was no strong correlation between the presence or absence of either one, as shown by the 2×2 analysis in Table 2. The 37 patients with HLA-DP antibodies were analyzed further, based on the data given in Table 3. It can be seen that 16 (43.2 %) of these patients (nos. 22–37) were awaiting retransplantation. This incidence was statistically significant higher ($P < 0.001$) than the fraction of HLA-DP antibody-positive patients awaiting their first transplant (Table 4).

This observation led us to retrospectively analyze the GFR of previous grafts and the HLA-DP antibody status in the 55 patients awaiting retransplantation. As demonstrated in Fig. 1, there was a higher rate of functioning kidney grafts in patients possessing HLA-DP antibodies before transplantation. The difference was statistically significant ($P < 0.025$) at day 500 post-grafting, but by day 1000 this difference had vanished.

Since the HLA-DP antibody census was done with serum samples drawn in April 1989, we followed up the 505 patients, 295 of whom were transplanted prior to

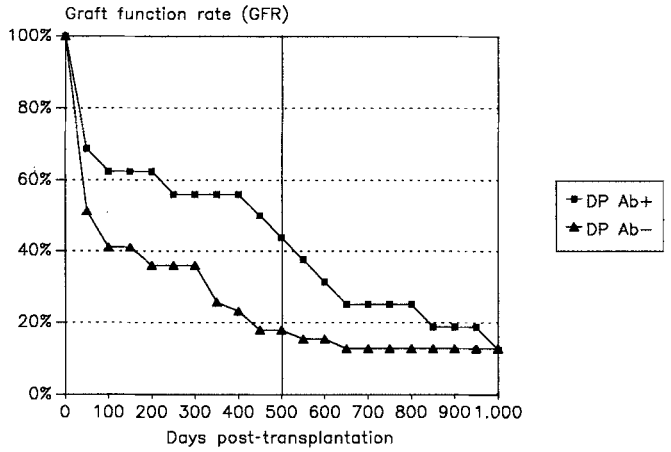

Fig. 1 Graft function rates in patients with preformed HLA-DP antibodies (■, $n = 16$) and without (▲, $n = 39$)

July 1992. The clinical course after transplantation with regard to irreversible rejection was recorded to find out whether there would be an influence of pre-existing HLA-DP antibodies. Even though the distribution of the individual HLA-DP antibody status compared to the incidence of kidney graft failure in 295 patients did not reach statistical significance, it is interesting to note that of the 16 patients with preformed HLA-DP antibodies, none lost his allograft during the period analyzed.

One patient without cytotoxic PRA was found to possess HLA-DP antibodies binding all of the reference LCL. Therefore, the patient's lymphocytes were EBV-transformed and tested in the MAILA as an autologous control. Also, the patient's LCL gave a definite, positive MAILA reaction, indicating the presence of HLA-DP autoantibodies of the IgG class. The patient was transplanted in July 1991 and his graft was still functioning in December 1993.

Discussion

For the precise evaluation of the complex role of preformed alloantibodies – especially HLA-specific ones – in renal transplantation, a thorough differentiation for antibody specificities and Ig classes is needed. HLA antibodies of the IgM class are reported to have no detrimental effect on graft survival, although these antibodies are able to cause cytotoxic reactions via complement activation [1, 13]. IgA antibodies to HLA even have a positive effect on graft survival, which is explained by their inability to activate complement while masking HLA determinants for other cytotoxic antibodies [6]. With regard to HLA antibodies of IgG and their subclasses, which are mainly responsible for the complement-dependent cytotoxicity in the graft, the IgG2 and IgG4 subclasses exhibit non- or weak complement binding.

The present study analyzed comparatively the incidence of classical complement-dependent lymphocytotoxicity versus the HLA-DP-specific binding capacity of antibodies in the serum of 505 patients awaiting kidney transplantation. The overall incidence of HLA-DP antibody-positive sera among the patients studied was 7.3 %. This figure is slightly lower than that observed with 9.7 % in pregnancy sera [9]. Only 54 % of the patients with such HLA-DP antibodies also demonstrated HLA class I-specific cytotoxic antibodies. Thus, there was no strong correlation between the production of the two antibody types. This could be due to the relatively low number of HLA-DP-positive T cells [2], representing approximately 80 % of the peripheral blood lymphocytes used for cytotoxic antibody screening and crossmatch procedures. Another explanation could be that the majority of HLA-DP antibodies are not cytotoxic but detectable from antigen-binding by the MAILA. The latter would be an argument to explain the unexpected finding that preformed HLA-DP antibodies had, at least for the first 500 days postgrafting, a beneficial effect on GFR. Since we assayed HLA-DP-specific IgG antibodies, their IgG2 or IgG4 subclasses may, like HLA antibodies of the IgA class, prevent the humoral or cellular cytotoxic attack mediated by other HLA antibodies by masking the respective target antigens. A noteworthy observation was the detection of one patient with HLA-DP autoantibodies of the IgG class without any obvious negative effect on the kidney graft.

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