Genotyping Analysis of HLA Class II Genes in Donor-Recipient Kidney Transplant Pairs

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THE CLINICAL outcome of kidney transplantation depends greatly on the HLA class II matching between the donor and the recipient. Identification of HLA class II specificities is usually performed on the basis of serology; however, serological typing has many drawbacks owing to factors that include cell viability and reagent quality, so allelic polymorphism sometimes remains undetectable by standard serology. The allelic polymorphism at different loci can be better determined using molecular biology techniques, such as sequence-specific oligonucleotides (SSO) typing and recently by sequence-specific primers (SSP).[1]

In this study we have retrospectively performed genomic HLA class II typing using PCR-SSO and PCR-SSP techniques in 17 cadaveric renal donor-recipient pairs, and we have correlated the occurrence of graft rejection within the first posttransplant year with regard to the mismatch grades of DRB1, DQA1, and DQB1 alleles.

MATERIALS AND METHODS

Study Subjects

Seventeen patients transplanted during the period from August 1988 to December 1991 and their cadaveric donors were entirely typed for HLA-DRB1, -DQA1, and -DQB1 by genomic typing, including PCR-SSO and PCR-SSP and by serological typing.

Diagnosis of Acute Rejection

The episodes of graft rejection were diagnosed both clinically and histologically. All episodes of rejection were successfully treated with high-dose steroids.

Serological Typing

Serological typing was performed by the NIH standard microlymphocytotoxicity assay and by the "two-color fluorescence" technique[2] using antisera-defining DR1-DRw10. The splits of DR2, DR3, and DR6 were not assigned (Table 1). Serological data were estimated using Lambda Scan Plus Software computer packages.

Genomic Typing

Genomic DNA from lysed leukocytes of cadaveric donors and transplant recipients and from frozen spleen tissues of donors was obtained by digestion with proteinase K[3] and extracted by standard methods of "salting out." The DNA samples were amplified[4,5] in a Gene Amp PCR System 9600 (Perkin-Elmer). The second exon of HLA-DQA1 was typed using GH26-GH27 biotinylated primers in 32 cycles of 3-step PCR amplification (94°C 1 minute; 60°C 30 seconds; 72°C 30 seconds). The HLA-DQB1 region was typed using DQBAMP-A and DQBAMP-B from XI Histocompatibility Workshop in 30 cycles of 3-step PCR amplification (96°C 1 minute; 55°C 45 seconds; 72°C 1 minute). HLA DRB1 region was typed by a generic amplification using DRBAMP-A and -B primers in 32 cycles of 3-step PCR amplification (95°C 1 minute; 57°C 1 minute; 72°C 1 minute).

The amplified DNA samples were dot-blotted on the nylon membranes. Alleles were identified by autoradiography after hybridization with 32P-ATP end-labeled SSO probes from XI Histocompatibility Workshop (19 SSOs for DRB1, 19 SSOs for DQB1), by DIG-ddUTP-labeled nonradioactive probes (DQβ-DRβ)[6,7] or by using 9 oligos in "reverse blotting" on Cetus strip (DQα).

All 17 kidney donor-recipient pairs were DRB1-typed by a "low resolution" PCR-SSP typing using 20 primer mixes: 17 for assigning DR1-DR18, 2 for identifying DR52 and DR53 superspecificities, and 1 for a negative control. Each PCR reaction mixture contained group-specific DRB primers and an internal positive control primer pair amplifying the third intron of DRB1 genes (796 bp fragment).[1]

Table 1. HLA DRB1, -DRB3, -DRB4 Allelic Definition

Serotyping vs Oligotyping		
HLA-DR Types by Serology	PCR-SSO	PCR-SSP
1	DRB1*01	DRB1*01
2	DRB1*02	DRB1*15,*16
3	DRB1*03	DRB1*0301,*0302
4	DRB1*04	DRB1*04
11 (5)	DRB1*11	DRB1*11
12 (5)	DRB1*12	DRB1*12
6	DRB1*13-14	DRB1*13,*14
7	DRB1*07	DRB1*07
8	DRB1*08	DRB1*08
9	DRB1*09	DRB1*0901
10	DRB1*10	DRB1*1001
52		DRB3*0101–0301
53	DRB4*0101	DRB4*0101

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Table 2. HLA-DRB1, -DRB3, -DRB4 Alleles: Serotyping vs Genotyping 17 Kidney Transplant Pairs

DR Types by Serology				DRB1 Alleles by PCR-SSO				DRB1 Alleles by PCR-SSP			
R		D		R		D		R		D	
3	7	3	7	03	07	03	07	0301	07	0301	07
7	11	3	7	07	11	03	07	07	11	0301	07
1	4	1	4	01	04	01	07	01	04	01	07
4	5	4	5	04	11	04	12	04	11	04	12
4	5	4	/	04	11	04	01	04	11	04	01
2	11	11	7	02	11	11	07	15	11	11	07
3	/	/	6	03	03	03	13	0301	0301	0301	13
11	/	11	/	11	11	11	08	11	11	11	08
1	2	1	2	01	02	01	02	01	15	01	15
2	11	2	/	02	11	02	02	16	11	16	16
2	4	2	6	02	04	02	13	15	04	16	13
11	6	11	6	11	06	11	14	11	X	11	14
10	6	3	6	10	14	03	14	10	14	0301	14
2	/	12	2	02	02	12	02	15	15	12	15
3	7	3	/	03	07	03	13	0301	07	0301	13
2	12	2	7	02	X	02	07	15	16	16	07
4	11	4	8	04	11	04	08	04	11	04	08

X = undetected DRB1 alleles.

RESULTS

Serological and genomic typing of the 17 kidney transplant pairs is shown in Table 2. Comparison of HLA-DR serological and genomic typing performed in transplant recipients and cadaveric donors showed a total concordance in antigen assignment in recipients, whereas in cadaveric donors the discrepancies were for both the molecular techniques and amounted to 29.4%.

Simultaneously, we analyzed 113 random healthy subjects as control group in evaluation of discrepancies between serotyping and oligotyping.

Results of the HLA-DR typing performed using PCR-SSO and PCR-SSP techniques correlated well, as previously observed from other authors,[8] but PCR-SSP showed a major degree of resolution, allowing us to define better the DRB1 subtypes. The discordant results (29.4%) consisted of DRB1 specificities undetected by serological typing (23.5%) and in incorrect assignment of DR alleles (5.9%).

The percent of disparities analyzed by PCR-SSO in subjects investigated as controls amounted to only 4.3%, 3.6% being alleles typed serologically as blanks and 0.8% alleles incorrectely typed. In cadaveric donors, the serological incorrect assignation of HLA-DR alleles regarded HLA-DR4, which genomic typing demonstrated to be DR7. The definition of blanks in this group regarded DR1, DR3, and some antigens difficult to type serologically, such as DR13 and DR8 (Table 1).

The allelic variability in the different HLA loci can be better defined using molecular biology techniques; in fact, serological typing was unable to efficiently split the allelic specificities, such as DR2 (15,16), DR3 (17,18), and some DR6 splits (Table 1). Moreover, genotyping allowed us to define the HLA homozygosity in 3 individuals and the HLA heterozygosity in the remaining subjects.

It is known that the appearance of episodes of rejection during early posttransplant months can affect the outcome of long-term transplant (reduction of about 20% of graft survival).[9] Out of 17 recipients examined, 6 showed episodes of rejection during the 1-year postoperative period (20 days, 7 months). All episodes of rejection were successfully treated with high-dose steroids.

The occurrence of graft rejection was correlated with the mismatch (MM) grades of DRB1, DQA1, and DQB1 alleles in kidney transplant pairs. As for DRB1 alleles, while serological typing evidenced a distribution of episodes of rejection among the *zero* mismatch (1/5) group, the *one* mismatch (5/11), and the *two* mismatch (0/1) patient group, actual genomic typing displayed a different distribution.

Patients with episodes of rejection were present in *one* mismatch (5/14) and *two* mismatch (1/1) groups (Fig 1). The possible implication of DQ compatibility (DQA1 and DQB1) in kidney transplant outcome was also evaluated. The distribution of rejections related to DR-DQ mismatch grade has shown that oligotyping allowed a wider spectrum of definition (*one* mismatch: 0/1; *two* mismatches: 1/5; *three* mismatches: 3/9; *four* mismatches: 1/1; *six* mismatches: 1/1) vs serotyping (*zero* mismatch: 1/5; *one* mismatch: 2/5; *two* mismatches: 3/6; *four* mismatches: 0/1) (Fig 2).

DISCUSSION

Knowledge of the great polymorphism of the HLA system is continuously increasing. The evaluation of HLA specificities in clinical application points out, however, the need

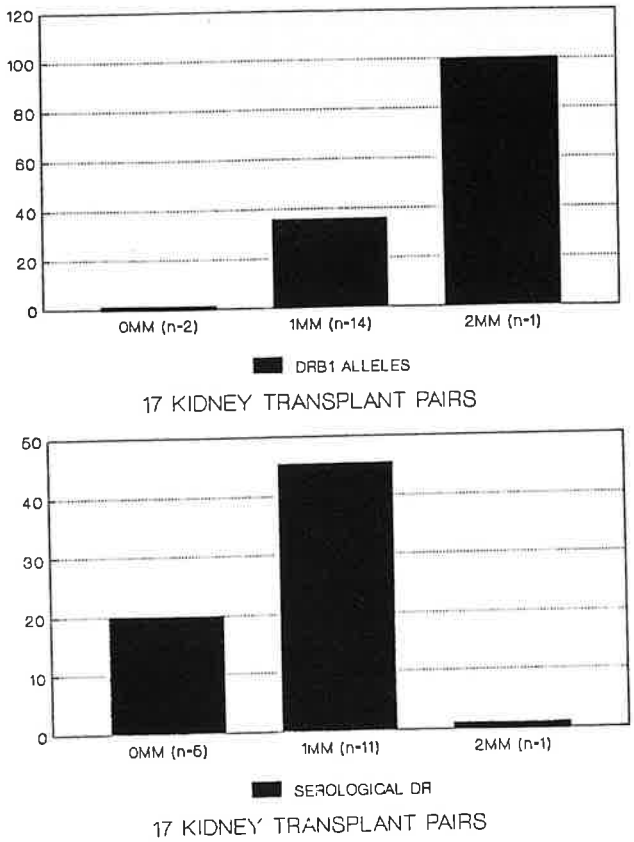

Fig 1. Serological-genomic DR matching and acute rejection (<1 year).

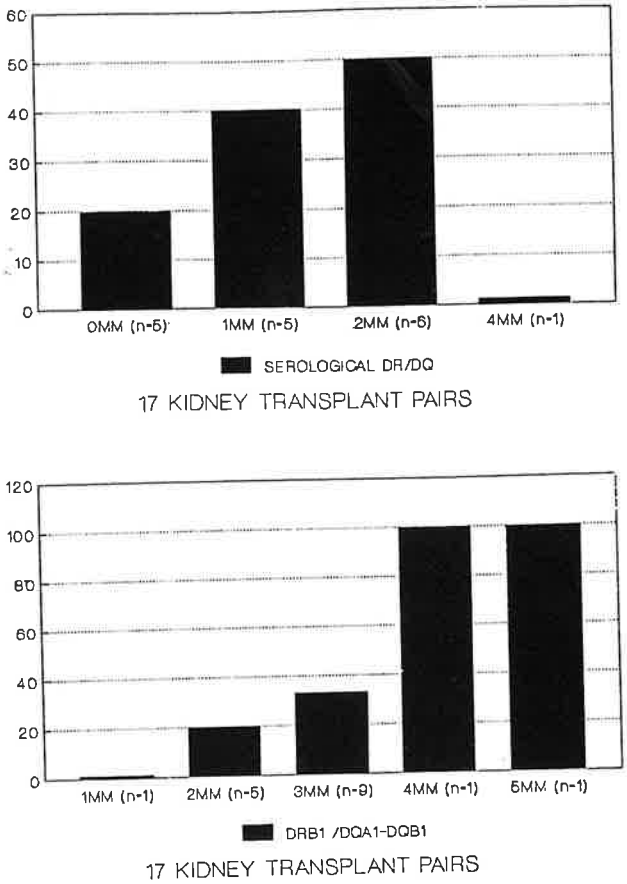

Fig 2. Serological-genomic DR/DQ matching and acute rejection (<1 year).

for an exact tissue typing.[10] Particularly, HLA class II matching has an important role in inhibiting the beginning of early episodes of rejection and consequently in the long-term graft survival. HLA class II antigen typing performed by serology is often inadequate owing to the lack of monospecific antisera, low expression of these antigens in the cell surface of T cells, poor viability of target cells, and cross-reactivity between alleles.[11]

The need for better methods to detect HLA class II polymorphism has led to the use of molecular biology techniques for HLA typing. Hence, we performed oligotyping to evaluate whether discrepancies between donor and recipient could affect the outcome of the graft. Discrepancies were found between DR antigens defined by serology and DRB1 alleles by genotyping, as previously reported by some authors.[11,12]

HLA-DR compatibility has a strong effect on long-term graft survival. In this study we observed that the discrepancies between serotyping and genotyping for DRB1 locus were particularly referred to cadaveric donors and consisted in serologically cross-reactive antigens, antigens known to be difficult to detect and some DRw52-associated antigens such as DR13, DR8, and DR3.

Discrepancies were particularly frequent among samples in which only one DR specificity had been detected by serology; in fact, molecular typing was able to detect the presence of a second allele and to define the homozygous forms, also on the basis of linkage disequilibrium existing among HLA-DRB1 and -DQA1 and -DQB1.[13]

Moreover, DNA typing is able to confirm the presence of splits of an antigen, turning out a more sensitive and accurate technique. As regards the correlation of degree of mismatches with the incidence of acute rejection, because the latter was proportional to the number of the HLA-DR, -DQ mismatches, we could conclude that genotyping is beneficial in reducing episodes of acute graft rejection.

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