死体腎移植のレシピエント選択基準としてHLA-DRB1遺伝子型適合の意義

小林孝彰，横山逸男，打田和治，折原明，高木弘

HLA-DRB1 MATCHING AS A RECIPIENT SELECTION CRITERION IN CADAVERIC RENAL TRANSPLANTATION

Takaaki Kobayashi,[1,2]
Itsuo Yokoyama,[2]
Kazuharu Uchida,[3]
Akira Orihara,[2]
Hiroshi Takagi[2]

Department of Surgery II, Nagoya University School of Medicine, Nagoya 466, Japan;
Department of Transplant Surgery, Nagoya Daini Red Cross Hospital, Nagoya 466, Japan.

Mailing address: Department of Surgery II
Nagoya University School of Medicine
65 Tsurumai-cho, Showa-ku,
Nagoya 466, Japan

[1]Address correspondence to : Takaaki Kobayashi, M.D., Department of Surgery II, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466 Japan.

[2]Department of Surgery II, Nagoya University School of Medicine.

[3]Department of Transplant Surgery, Nagoya Daini Red Cross Hospital.

*Abbreviations : CsA, Cyclosporine A;
MLR, mixed lymphocyte reaction;
PCR, polymerase chain reaction;
RFLP, restriction fragment length polymorphism.

ABSTRACT

We retrospectively examined the effect of HLA-DRB1 matching at the DNA level compared with serological HLA-DR matching on acute rejection and graft survival in the patients who underwent primary cadaveric renal transplantation. For the patients with serological HLA-DR zero mismatch, the incidence of acute rejection in patients with DRB1 zero mismatch (3/20; 15 %) was significantly lower than in those with one or two DRB1 mismatches (10/21; 48 %). Five year graft survival in patients with DRB1 zero mismatch was 100 %, whereas that in those with one or two DRB1 mismatches was 76 %, although the difference was not statistically signigicant.

The fact that HLA-DRB1 matching at the DNA level influenced on the incidence of graft rejection after cadaveric renal transplantation is analogous to that found in the previous authors' study in living-related renal transplantation.

In conclusion, it is suggested that avoidance of mismatching for DRB1 alleles at the DNA level in recipient selection of cadaveric renal transplantation leads to an improvement of graft outcome.

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INTRODUCTION

Although the clinical value of HLA matching in Cyclosporine A (CsA)-treated renal transplantation remains controversial (1-6), it has been suggested that HLA-DR antigens exert a stronger influence on allograft survival than either HLA-A or B antigens by most of studies (7-11).

With the aid of DNA typing of HLA-DR antigens, it has been revealed that approximately a quarter of serologically determined HLA-DR typings were discrepant with those determined by DNA typing (12,13). Elimination of these discrepancies of HLA-DR mismatches by DNA typing would certainly lead to a theoretical benefit in the clinical renal transplantation (14). Moreover, by DNA typing it has become possible to analyze the extensive polymorphism of HLA-DR antigens at the DNA level (15,16).

We have previously reported that in living-related renal transplantation with one haplotype match, significantly fewer acute rejection episodes were seen in patients with zero mismatch for both serological DR and DRB1 at the DNA level than in those with DR zero mismatch and DRB1 one mismatch (17). The mismatching for DRB1 alleles related with incompatible Dw specificities defined by mixed lymphocyte reaction (MLR) (18) was a potent immunologic factor on the incidence of acute rejection in serological DR zero mismatched transplants.

In this article, we examined an effect of DRB1 matching at the DNA level on the outcome of primary cadaveric renal transplantation and a possibility of DRB1 matching as a

distinctive recipient selection criterion in cadaveric renal transplantation was indicated.

PATIENTS AND METHODS

Patients and immunosuppressive regimen. Between December, 1982 and December, 1991, 109 patients underwent primary cadaveric renal transplantation at the Nagoya Daini Red Cross Hospital and the Nagoya University Hospital. In order to examine the immunologic effect of HLA, 11 with graft failure secondary to non-immunologic causes such as primary graft non-function, infection or technical complications within three months posttransplant period were excluded, thus leaving 98 patients for this study. They were 73 males and 25 females. The mean age of the recipients was 36.8±8.9 (±S.D.) years old and that of the donors was 39.7±16.0. All of the patients received crossmatch-negative kidneys. A combination therapy with low dose of CsA, azathioprine and predonisolone was given to all of the patients as a basic immunosuppression. When postoperative serum creatinine reached below 3 mg/dl, azathioprine was discontinued and CsA dose was adjusted according to the whole blood level measured by high performance liquid chromatography.

Diagnosis of acute rejection. Clinical records on all of the patients were reviewed for episodes of graft rejection during the 3 months posttransplant period. The diagnosis of acute rejection was made both clinically and histologically. All of the patients with acute rejections were treated with high-dose steroids initially. For the patients with steroid-resistant rejections additional treatment with murine anti-CD3 monoclonal antibody (OKT3) or 15-Deoxyspergualin (DSG) was used.

Serological typing. All transplant recipients and donors were typed by microlymphotoxicity test using well standardized alloantisera. In the recipient selection, the priority was placed in HLA-DR matching status. Thus, the number of serological DR matching was zero mismatch in 63, one mismatch in 34 and two mismatches in one patient.

DNA typing. DNA samples of the recipients were extracted from peripheral blood lymphocytes and those of the donors from preserved lymphocytes or frozen tissue of one hour posttransplant biopsy. The second exon of HLA-DRB1 gene was amplified by polymerase chain reaction (PCR) from genomic DNA using group-specific primers (19). The procedure of PCR-restriction fragment length polymorphism (RFLP) method used has been previously described in detail (17).

The chi-square test was used for the comparisons between the groups. Graft survival was calculated by the method of Kaplan-Meier.

RESULTS

Serological HLA-DR types and PCR-RFLP defined DRB1 alleles of the recipients and the donors with serological DR zero mismatch are shown in Table 1. Discrepancy between serological and DNA typing was observed in 8 patients. In 14 patients, DNA typing could not be done because the specimens were not available.

The influence of DRB1 matching at the DNA level compared with serological DR matching on the incidence of acute rejection and graft survival rates is shown in Tables 2 and 3. Acute rejection episodes within 3 months posttransplant period were seen in 21 patients (33 %) with serological DR zero mismatch, whereas they were seen in 14 patients (41 %) with DR one mismatch. Among the patients with serological DR zero mismatch, acute rejection episodes were seen in 3 of 20 patients (15 %) with DRB1 zero mismatch, which were significantly lower than in those with one or two DRB1 mismatches (10 of 21 patients; 48 %).

The graft survival at 5 years excluding the graft losses due to non-immunologic causes was 90 % in patients with serological DR zero mismatch and 75 % in those with DR one mismatch. Among the patients with serological DR zero mismatch, the 5 year graft survival was 100 % in patients with DRB1 zero mismatch.

DRB1 mismatching and number of HLA-A, B mismatches are shown in Table 4. In 20 patients with DRB1 zero mismatch, 13 had one or two HLA-A mismatches and 13 had one or two HLA-B mismatches. Only 2 of each batch of the patients had the episode of acute

rejection.

DISCUSSION

Since the introduction of CsA, a significant progress has been made in the graft survival of cadaveric renal transplantation. Further progress was made possible by selecting HLA-A, B and DR zero mismatched recipients, although only a small number of patients benefited from it. In the present study we examined the significance of the matching for HLA-DRB1 alleles, which correlated with Dw specificity based on MLR, compared with that for serological HLA-DR. Significantly fewer acute rejection episodes were observed in patients with DRB1 zero mismatch than in those with one or two DRB1 mismatches. Although no significant difference in graft survival was noted probably because of the small number of the cases, the transplants with DRB1 zero mismatch showed a tendency of better graft survival than those with the counterpart.

The beneficial effect of zero mismatch for HLA-A, B, DR or B, DR can be explained by the fact that DRB1 alleles at the DNA level are concomitantly matched as a result of linkage disequilibrium. Indeed, more than a half of the patients with DRB1 zero mismatch had some HLA-A or B mismatches, but they were associated with a relatively low incidence of acute rejection. There is a possibility that the mismatching for HLA-class I antigens is of little clinical significance on acute rejection as long as DRB1 alleles are matched.

Opelz et al showed the value of DNA-matching at the serological level (DR1 to DR10) thereby eliminating DR typing

errors in the prediction of the graft outcome (14). In this study, DR antigens were classified further into details at the DRB1 allelic level. We thus, confirmed the value of DRB1 matching which influenced on the incidence of acute rejection. It is conceivable that better DRB1 matching is associated with improved result in renal transplantation. Of practical importance is a question whether the matching should be done at the DRB1 DNA level or is sufficient at the serological splits level. More experience is required to investigate the value of DRB1 matching in clinical transplantation.

It can be concluded that the clinical application of DNA typing coupled with conventional serological HLA typing provides an important information of the outcome of cadaveric renal transplantation. DNA typing by PCR-RFLP requires only 8 hours to complete DRB1 typing (15). It should be stressed that avoidance of mismatching for DRB1 alleles at the DNA level by performing DNA typing for recipient selection in advance can lead to an improvement of graft outcome in cadaveric renal transplantation.

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Table 1 HLA-DRB1 alleles defined by PCR-RFLP and acute rejection in primary cadaveric renal transplants with serological HLA-DR zero mismatch

Transplant No.	No. of Mismatches HLA-A,	HLA-B	HLA-DR Types (Serology) Recipient		Donor		HLA-DRB1 Alleles (DNA) Recipient		Donor		No. of HLA-DRB1 Mismatches	Acute Rejection
CD- 29	2	1	4	6	4	-	0405	1301	0405	0405[a]	0	−
CD- 49	0	0	1	6	1	6	0101	1302	0101	1302	0	−
CD- 50	0	0	2	-	2	-	1502	1501	1502	1502	0	−
CD- 62	1	1	9	-	9	-	0901	0901[a]	0901	0901[a]	0	−
CD- 67	0	1	1	8	1	-	0101	0803	0101	0101[a]	0	−
CD- 83	0	1	2	9	2	9	1502	0901	1502	0901	0	−
CD- 87	1	0	6	9	6	9	1302	0901	1302	0901	0	−
CD- 94	0	0	2	-	2	-	1501	1502	1502	1502[a]	0	−
CD-106	1	1	6	9	6	-	1401	0901	1401	1401[a]	0	−
CD-117	1	1	4	9	9	-	0405	0901	0901	0901[a]	0	−
CD-125	1	1	2	9	2	9	1502	0901	1502	0901	0	−
CD-127	2	0	1	9	1	9	0101	0901	0101	0901	0	−
CD-128	1	0	1	4	1	4	0101	0405	0101	0405	0	−
CD-129	1	1	4	5	4	-	0405	1101	0405	0405[a]	0	−
CD-135	1	2	2	9	2	9	1501	0901	1501	0901	0	−
CD-136	0	1	1	9	1	9	0101	0901	0101	0901	0	−
CD-139	1	1	2	6	2	6	1502	1302	1502	1302	0	−
CD- 66	1	0	2	-[b]	2	-	1502	1405	1502	1502[a]	0	+
CD- 68	0	1	1	6	1	-	0101	1302	0101	0101[a]	0	+
CD- 99	1	1	4	9	4	9	0405	0901	0405	0901	0	+
CD- 25	1	2	2	8	2	8	1501	0803	1501	0802	1	−
CD- 88	0	2	4	9	4	9	0403	0901	0401	0901	1	−
CD- 92	0	0	2	4	2	-	1502	0405	1502	1501	1	−
CD-107	1	1	4	8	4	8	0406	0803	0405	0803	1	−
CD-112	1	0	4	5	4	5	0405	1101	0407	1101	1	−
CD-114	1	1	4	6	4	-	0406	1405	0406	0405	1	−
CD-121	0	1	6	9	6	-	1401	0901	1401	1405	1	−
CD-140	1	0	4	6	4	6	0406	1302	0403	1302	1	−
CD- 37	1	1	2	4	2	4	1501	0405	1501	0406	1	+
CD- 65	0	1	4	6	4	6	0405	1302	0403	1302	1	+
CD- 72	1	1	2	9	2	-	1502	0901	1502	1501	1	+
CD- 86	1	1	6	9	6	9	1301	0901	1302	0901	1	+
CD-101	0	1	4	8	4	8	0405	0803	0405	0802	1	+
CD-116	1	1	5	6	5	6	1202	1401	1201	1401	1	+
CD- 60	1	1	2	6	2	-	1502	1405	1501	1601	2	−
CD-122	1	1	8	-[b]	8	-[b]	0802	1401	0803	1405	2	−
CD-133	1	1	2	6	6	-	1502	1302	1301	1402	2	−
CD- 85	0	2	6	8	6	8	1401	0802	1302	0803	2	+
CD-111	1	1	6	9	6	-	1405	0901	1401	1302	2	+
CD-123	1	2	4	5	4	5	0406	1201	0405	1202	2	+
CD-134	0	2	5	6	5[b]	6	1101	1302	1403	1401	2	+
CD- 36	0	2	2	9	9	-[b]	1502	0901	0901	1201	Discrepant	−
CD- 38	0	0	4	6	4	6[b]	0410	1302	0405	0803	Discrepant	−
CD- 96	1	1	5	-[b]	5	-[b]	1202	1403	1102	0701	Discrepant	−
CD-113	1	2	2	5[b]	2	5	1501	1403	1501	1201	Discrepant	−
CD- 78	1	1	2	5[b]	2	5	1502	1402	1502	1202	Discrepant	+
CD-105	0	0	2	-	2	-[b]	1502	1501	1502	1403	Discrepant	+
CD-119	0	0	2	9	2	-[b]	1502	0901	1502	1201	Discrepant	+
CD-142	1	1	2	5[b]	5	-[b]	1501	1402	1101	0802	Discrepant	+

[a] In serological DR "blank" alleles, it was confirmed that no amplified DNA was obtained by any primers other than the specific primer of DR antigen indicated.

[b] HLA-DRB1 allele assigned by PCR-RFLP was discrepant with serologically defined HLA-DR type.

Table 2. Influence of DRB1 (DNA) matching compared with DR (serology) matching on the incidence of acute rejection in primary cadaveric renal transplantation

No. of DR Mismatches (Serology)	Incidence of Acute Rejection (%)	No. of DRB1 Mismatches (DNA)	Incidence of Acute Rejection (%)
0	21／63 (33 %)	0	3／20 (15 %) [ab]
		1 ～ 2	10／21 (48 %) [a]
1	14／34 (41 %) [b]		

[a b] $P<0.05$

Table 3. Influence of DRB1 (DNA) matching compared with DR (serology) matching on graft survival without non-immunologic losses in primary cadaveric renal transplantation

No. of DR Mismatches (Serology)	Graft Survival (%)		No. of DRB1 Mismatches (DNA)	Graft Survival (%)	
	1 Year	5 Year		1 Year	5 Year
0 (n=63)	97	90	0 (n=20)	100	100 [a]
			1 ~ 2 (n=21)	95	76
1 (n=34)	91	75 [a]			

[a] $P < 0.05$

Table 4. DRB1 mismatching and number of HLA-A, B mismatches in primary cadaveric renal transplantation

No. of DR Mismatches (Serology)	No. of DRB1 Mismatches (DNA)	HLA-A Mismatches		HLA-B Mismatches	
		0	1～2	0	1～2
0	0 (n=20)	7 (1)	13 (2)	7 (1)	13 (2)
0	1～2 (n=21)	7 (4)	14 (6)	3 (0)	18 (10)
1	1～2 (n=34)	16 (9)	18 (5)	5 (3)	29 (11)

( ) : Acute Rejection