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The significant effect of HLA-DRB1 matching on acute rejection in kidney transplants

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Abstract The object of the present study was to confirm the HLA-DRB1 matching effect on rejection crisis, its severity, and kidney graft survival based on genotyping. Ninety-four renal allografts were included in this study. DNA typing of HLA-DRB1 was performed by the polymerase chain reaction sequence-specific oligonucleotide method. The incidence of acute rejection within 6 months following transplantation, the frequency of OKT3 administration for steroid-resistant rejection, histopathological findings, and graft survival rate were compared between the DRB1-matched (n = 23) and DRB1-mismatched (n = 71) groups. Four acute rejections occurred in the DRB1-matched group (incidence; 17 %) and 40 in the DRB1-mismatched group (56 %). In the DRB1-matched group, the incidence of acute rejection was significantly less frequent than that of the DRB1-mismatched group ($P < 0.005$). In the DRB1-matched group, only one patient received OKT3 administration (4 %), in contrast to 16 of 71 patients in the DRB1-mismatched group (23 %). The use of OKT3 was significantly less frequent in the DRB1-matched group ($P < 0.05$). Histopathological findings from biopsy specimens showed no constant distribution of pathological grades of acute rejection according to DRB1 matching in the present study. The graft survival rate in the two groups did not differ significantly, but the graft survival rate in the DRB1-mismatched group had a tendency to decrease as the grafts survived longer. In conclusion, the results of the present study confirm that HLA-DRB1 matching has marked beneficial effects on kidney transplants through reduction of the acute rejection rate and decrease of the severity of rejection, and suggest that improvement of graft survival will be obtained through kidney allocation to a DRB1-matched recipient.

Key words Kidney transplantation · HLA-DRB1 matching · Acute rejection

Introduction

Rejection is the most serious factor affecting kidney graft survival. Gulanikar et al. [3] reported that acute rejection affected long-term kidney graft survival. On the other hand, Nankivell et al. [15] demonstrated that HLA-DR mismatch and the presence of vascular rejection were the most important predictors of the severity of rejection. Some reports have shown a significantly lower incidence of acute rejection in HLA-DRB1-compatible grafts than in DRB1-incompatible grafts, both in living-related and cadaver cases [11, 12, 20]. Our previous reports showed that linkage disequilibria between HLA-B and DRB1 were so strong that HLA-DRB1 could be inferred in Japanese donor-recipient pairs according to the two locus associations [5, 10]. The infer-

red HLA-DRB1 matching had a critical effect on long-term kidney graft outcome [6–8]. The results revealed no difference in kidney graft survival between HLA-identical siblings and zero-mismatch for HLA-DRB1 in living-related and cadaver donor transplants [6, 7]. These suggested that the 4-antigen and 6-antigen match effects as a result of the locus associations within HLA alleles.

The object of the present study was to confirm the HLA-DRB1 matching effect on rejection crisis, its severity, and kidney graft survival based on genotyping.

Materials and methods

HLA typing

In all of the recipients and donors included in this study, HLA class II typing was performed by two methods, serotyping and genotyping. Conventional serological typing was performed with a complement-dependent microcytotoxicity test using well-standardized alloantisera. DNA typing of HLA-DRB1 was performed by polymerase chain reaction with the sequence-specific oligonucleotide (PCR-SSO) method. Reference protocols were as reported previously [4, 5]. A brief protocol of procedures for this study is presented here.

DNA was extracted and precipitated after the lysis of the tissue cellular component. Genomic DNA was amplified with Taq DNA polymerase and primers in a DNA thermal cycler. The amplified DNA was spotted and hybridized with digoxigenin-11-dUTP-labelled SSO probes, which were determined at the 11th International HLA Workshop [1]. Thirty-one HLA-DRB1 alleles were found in the previous study on 916 Japanese individuals [5].

Patient and immunosuppression

Ninety-four renal allografts performed at Hyogo College of Medicine and Hyogo Prefectural Nishinomiya Hospital were included in this study. Fifty-eight were engrafted from living-related donors and 36 were from cadaver donors. There were 46 males and 48 females. The mean age was 33.5 ± 1.4 (± SE) years. The mean age of the donors was 49.4 ± 1.7 (± SE) years. The mean follow-up period after transplantation was 59.7 ± 6.0 months. Cyclosporine-based immunosuppression was conducted in 71 patients, and azathioprine-based immunosuppression and tacrolimus (FK506)-based immunosuppression were conducted in 11 and 12 patients, respectively. Steroid was given to all patients.

According to the genotyped DRB1 matching, 94 patients were divided into two groups, zero mismatch for DRB1 (n = 23) and 1 or 2 mismatches for DRB1 (n = 71). Age and gender of the recipients, age of the donors, posttransplant follow-up period, and immunosuppressive regimens were compared between the two groups. Characteristics of each group are summarized in Table 1.

Diagnosis and treatment of acute rejection

A diagnosis of acute rejection was confirmed from both the clinical and histopathological findings. The clinical parameters were as follows; 25 % reduction in renal function, graft tenderness or swelling, fever, proteinuria, increase in urine-FDP or in -NAG, decrease of urine volume. Core needle biopsy was performed in 37 cases, especially in the case to whom OKT3 was administered. Other non-immunological events causing renal dysfunction, such as drug nephrotoxicity, infection, or surgical complications, were ruled out by the histopathological, radiological, and/or microbiological findings. Acute rejection was ruled out in 9 cases by histopathological diagnosis: 5 with nephrotoxicity from FK506, 1 with nephrotoxicity from cyclosporine, 1 with acute tubular necrosis, 1 with glomerulonephritis, and 1 with hemolytic uremic syndrome, respectively. The severity of acute rejection was classified by histopathology and the kinds of anti-rejection therapies. Histopathologically, acute rejections were classified as follows: mild interstitial acute cellular rejection (grade I AR), moderate interstitial acute cellular rejection (grade IIA AR), moderate interstitial acute cellular rejection with vascular component (grade IIB AR), and severe vascular rejection (grade III AR) according to the Banff working classification [21].

Intravenous high doses of pulse steroids and OKT3 were administered as anti-rejection therapies. Pulse steroids were used initially in patients at their first acute rejection, whereas OKT3 was used in patients with more severe and steroid-resistant acute rejection. The incidence of acute rejection during the first 6 months following transplantation and the time from transplantation to the onset of the first acute rejection were compared between the DRB1-matched and DRB1-mismatched groups in this study. Cases with severe rejection in whom OKT3 had been administered were also compared. Histopathological analysis of the grade of acute rejection was carried out. Statistical significance was evaluated by the chi-squared test.

Graft survival rate

The graft survival rate was calculated on 31 August 1995 by Kaplan-Meier's method. Patient death or return to hemodialysis was regarded as a graft failure. The Cox-Mantel test was used to evaluate the statistical significance.

Results

Characteristics of patients according to matching or mismatching are shown in Table 1. The groups were compatible in recipient age, donor age, gender distribution, the rate of living-related donors, and the posttransplant follow-up period, with the exception of the frequency of cyclosporine use. In the DRB1-matched group, the use of cyclosporine was less frequent ($P < 0.05$): 13 of 23 patients received cyclosporine, in contrast to 58 of 71 in the DRB1-mismatched group.

Acute rejection

Table 2 shows the incidence of acute rejection and the use of OKT3 in each group. Intravenous pulse steroids were given initially to all patients with acute rejection. Forty-four acute rejection episodes were observed in 94 patients. There were four acute rejection episodes in the DRB1-matched group (17 %) and 40 in the DRB1-mismatched group (56 %). In the DRB1-matched group, the incidence of acute rejection was significantly less frequent than that of the DRB1-mismatched group

Table 1 Characteristics of the groups with and without HLA-DRB1 matching

	Group		P
	DRB1-matched (n = 23)	DRB1-mismatched (n = 71)	
Recipient age (years)[a]	33.4 ± 2.0	33.5 ± 1.2	NS
Donor age (years)[a]	47.5 ± 2.0	50.0 ± 1.7	NS
Male	11 (48 %)	35 (49 %)	NS
Living-related grafts	15 (65 %)	43 (61 %)	NS
Follow-up months[a]	62.3 ± 9.8	58.9 ± 4.8	NS
Main immunosuppressant			
Azathioprine	5 (22 %)	6 (8 %)	NS
Cyclosporine	13 (57 %)	58 (82 %)	< 0.05
FK506	5 (22 %)	7 (10 %)	NS

[a] Mean ± SE

Table 2 Incidence, severity, and timing of acute rejection according to DRB1 matching

	Group		P
	DRB1-matched (n = 23)	DRB1-mismatched (n = 71)	
Patients with acute rejection	4 (17 %)	40 (56 %)	< 0.03
OKT3 administration	1 (4 %)	16 (23 %)	< 0.05
Days from transplant to rejection[a]	37.3 ± 12.3	38.7 ± 6.8	NS

[a] Mean ± SE

Table 3 Histopathological diagnosis of acute rejection, classified according to Banff working classification

	Group	
	DRB1-matched (n = 2)	DRB1-mismatched (n = 25)
Grade I (mild interstitial rejection)	0	16
Grade IIA (moderate interstitial rejection)	1	8
Grade IIB (moderate interstitial rejection with vascular component)	1	1

($P < 0.005$). Mean time from transplantation to onset of the first acute rejection episode was 38.4 ± 6.2 days (Table 2). Acute rejection occurred at 37.3 ± 12.3 days after transplantation on average in the DRB1-matched group and 38.7 ± 6.8 days in the DRB1-mismatched group. There was no significant difference in the time between the two groups.

OKT3 treatment

Seventeen of 94 patients (18 %) received OKT3 as rescue therapy for steroid-resistant acute rejection (Table 2). In the DRB1-matched group, only 1 patient received OKT3 administration (4 %), in contrast to 16 of 71 patients in the DRB1-mismatched group (23 %). The use of OKT3 was significantly less frequent in the DRB1-matched group than in the DRB1-mismatched group ($P < 0.05$).

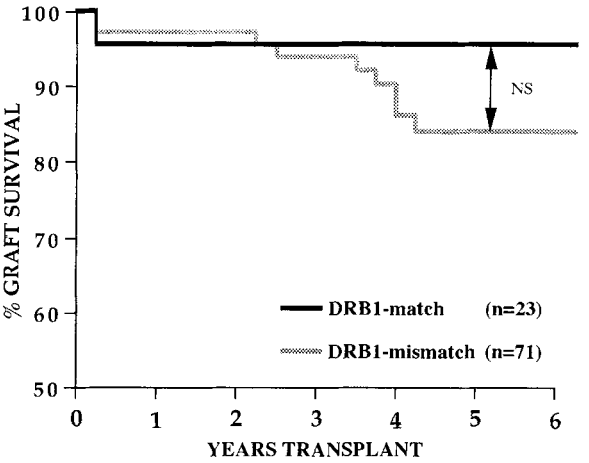

Fig. 1 Graft survival rate in years after transplantation is shown according to genotyped HLA-DRB1 compatibility

Histopathological diagnosis

A biopsy specimen was taken in 37 of 94 patients. Twenty-seven of these 37 biopsies were included in this study. The histopathological diagnoses of the patients with acute rejection are shown in Table 3. In the DRB1-matched group, 2 biopsy specimens were diagnosed as acute rejection, 1 as moderate interstitial cellular rejection (grade IIA AR) and 1 as moderate interstitial cellular rejection with vascular component (grade IIB AR). On the other hand, 25 cases were diagnosed as acute rejection in the DRB1-mismatched group. There were 16 cases of mild interstitial cellular rejection (grade I AR) observed in the DRB1-mismatched group. More severe acute rejection was found in 9 of the DRB1-mismatched patients, 8 cases were grade IIA AR and 1 was grade IIB AR. No severe vascular rejection (grade III AR) was observed in either group.

Graft survival rate

The overall graft survival rate is shown in Fig. 1. The graft survival rate at 3 years was almost the same in the two groups: 96 % in the DRB1-matched group and 94 % in the DRB1-mismatched group. Patients with a DRB1-matched graft had a 12 % higher survival rate at 5 years than patients with a DRB1-mismatched graft (96 % vs 84 %). Although there was no significant differ-

ence in the graft survival between the two groups, the difference in the graft survival rate between the DRB1-matched group and DRB1-mismatched group gradually increased.

Discussion

Acute rejection is an important cause of graft dysfunction in the early posttransplant period and has been correlated with permanent graft impairment, graft loss, and decreased graft survival [2, 9, 13, 23]. In the serological study, the severity of acute rejection was associated with the matching grade of HLA-DR [15]. It has been reported that the incidence of acute rejection in HLA-DRB1-matched patients was less than that in DRB1-mismatched patients [11, 12, 20]. The significance of DRB1 matching in renal transplantation has been discussed because of its precision and specificity [17, 22]. To assess the effect of DRB1 matching on long-term kidney graft outcome, we previously reported that DRB1 alleles could be inferred from the linkage disequilibrium between HLA-B antigens and DRB1 alleles [5, 10]. In the inference study, the graft survival rate of HLA-DRB1-matched patients was almost the same as in HLA-identical siblings and was significantly higher than that of HLA-DRB1-mismatched cases. In contrast, the HLA-DRB1-mismatched group had nearly the same success rate as the serologically HLA-DR mismatched group. In our analysis of 511 kidney transplants, the 5-year graft success rate was 94 % in the DRB1-matched group, compared to 73 % in the DRB1-mismatched group [6–8]. These results are supported by the fact that the number of genomic HLA-DR mismatches in long-term survivors were significantly less than in recent transplants [19]. A retrospective study on the effect of genotyped DRB1 matching on graft survival has also been reported [16]. This benefit of DRB1 matching has been attributed to a lower rejection rate than occurs with DRB1-mismatched grafts [11, 12, 20]. In the present study, the incidence and the time of onset of acute rejection, the frequency of OKT3 administration which was used to treat severe and steroid-resistant acute rejection, the severity of rejection according to the histopathological diagnosis, and the graft survival rate were compared between the DRB1-matched and DRB1-mismatched groups. Our study has revealed that the incidence of acute rejection episodes was significantly less in the DRB1-matched group than in the DRB1-mismatched group ($P < 0.005$). Furthermore, the frequency of use of OKT3, which has been used only as rescue treatment for steroid-resistant acute rejection in our institute, was also significantly less in the DRB1-matched transplants ($P < 0.05$). These results demonstrate that DRB1 matching has beneficial effects not only in decreasing the incidence of acute rejection, but also in decreasing severe rejection. Nankivell et al. [15] reported that the increase in the rate of acute rejection and the greater severity of renal dysfunction with each acute rejection episode were effects derived from increased HLA-DR mismatch. Our data support the notion that this correlation of HLA-DR matching and acute rejection could be emphasized in an HLA-DR genotyped study.

On the other hand, histopathological findings from biopsy specimens showed no constant distribution of pathological grades of acute rejection according to DRB1 matching in the present study. The lack of correlation between HLA-DR mismatch and the degree of cellular infiltration on biopsy during rejection was seen in other studies [14, 15]. Although a larger number of cases is required to draw a conclusion on the correlation between DRB1 matching and histopathological characteristics, it is noteworthy that a few moderately interstitial rejections were observed even in the DRB1-matched transplants.

Graft survival rate in the DRB1-matched group and the DRB1-mismatched group did not differ significantly, but the graft survival rate in the DRB1-mismatched group had a tendency to decrease as the grafts survived longer. The reason why the result of the genotyped study was less clear compared with that in the inferred study may be that about two-thirds of the cases were genotyped retrospectively, so that patients losing grafts in the early posttransplant period were not included in the analysis. Many of these patients, were assumed to be mismatched for DRB1 based on their serology. A recent report indicates that the genotyped HLA-DR antigen matching has significant effect on graft survival, but no more effect than with further split-typing of HLA-DR in genomic DNA typing [18]. Our study suggests that the benefit of compatibility for HLA class II at the DNA level can be derived from HLA-DRB1 typing because of its specificity and precision.

In conclusion, the results of the present study confirm that HLA-DRB1 matching has marked beneficial effects on kidney transplants through the reduction of the acute rejection rate and the decrease of the severity of rejection, and suggest that improvement of graft survival will be obtained through kidney allocation to a DRB1-matched recipient.

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