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The Impact of HLA-A Matching in Corneal Transplantation

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Previously, we have reported the results of our retrospective study on the effect of HLA class II allele matching on the outcome of corneal transplant. Here, we demonstrate our findings of the study for HLA class I allele matching in the same study subjects. Eighty transplant recipients were typed for HLA-A, and 79 transplant recipients were typed for HLA-B alleles, by PCR-SSOP. The association between HLA class I allele matching and 1-year rejection-free graft survival was evaluated. When a total of 79 transplant recipients were subdivided into groups with matching (one to four alleles matched) and without matching (no allele matched) for HLA class I (HLA-A and -B), a significantly higher rate of 1-year rejection-free graft survival was detected in transplant recipients with matching, compared with those without matching (p=0.0258). We have found that matching for at least one HLA class I allele was more beneficial especially in high-risk transplant recipients (p=0.0076). Also, an analysis of matching for each locus separately, detected that, HLA-A matching was significantly associated with a higher rate of 1-year rejection-free graft survival. Transplant recipients with HLA-A matching (one or two-alleles matched) had significantly higher rejection-free graft survival compared with those without matching (no allele matched), when high- and low-risk groups were analyzed together (p=0.0099). Furthermore, matching for HLA-A allele was significantly beneficial compared with no matching in high-risk transplant recipients (p=0.0154). Nevertheless, no significant effect of HLA-B matching was detected. We conclude that HLA class I, especially HLA-A matching has a beneficial effect for corneal transplant outcome.

Keywords : HLA class I genotyping, Rejection-free graft survival, HLA-A matching

INTRODUCTION

Corneal graft outcome is measured in terms of graft survival as with other organ transplantations and immunological rejection represents the main cause of graft failure. Possible benefits of HLA matching on corneal graft outcome have been studied for many years without a consensus being obtained. Several groups have found beneficial effects of HLA matching [1-12], while others have found no effect [13-15]. However, in most of these studies, HLA-A, -B, -DR compatibility was determined by conventional serological typing [1-9, 13-15].

In order to prove the clinical importance of HLA matching in organ transplantation, more reliable techniques which are able to provide an accurate determination of so-called broad specificities or antigen splits are required. Molecular biology contributed to the development of such up-graded methods. The PCR (polymerase chain reaction) based DNA typing methods, such as PCR-SSOP (PCR with sequence-specific oligonucleotide probes), PCR-RFLP (PCR with restriction fragment length polymorphism), PCR-SSP (PCR with sequence-specific primers), PCR-MPH (PCR with microtiter plate hybridization), ARMS/PCR-SSP (amplification refractory mutation system), nested PCR-RFLP, etc. has already been successfully applied to

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HLA genotyping [16-24]. An application of DNA typing techniques to transplant recipients revealed that, approximately one fourth of HLA-DR antigens determined by conventional serological typing, were discrepant with those determined by DNA typing, and this high discrepancy rate may be partially responsible for unexpected transplant failures [25-29]. Differences at the molecular level, that could not be detected serologically, could lead to graft rejection.

With the purpose to evaluate the actual influence of HLA matching on corneal graft outcome, we performed a retrospective antigen matching study using DNA typing techniques. Previously we have reported the results of our study on the effect of HLA class II allele matching, which detected a strong association between HLA-DPB1 matching and rejection-free graft survival in high-risk corneal transplantation [10, 12]. In the present study, we analyzed the association between HLA class I allele matching and 1-year rejection-free graft survival by retrospective PCR-SSOP typing.

MATERIALS AND METHODS

STUDY SUBJECTS

A retrospective antigen matching study began in January 1991 for penetrating keratoplasty and limbal allograft recipients, who had received corneal allografts and had been followed up at the Department of Ophthalmology, Tokyo Dental College. Study subjects had been described in detail in our previous report [12]. They were grouped into high and low risk groups for allograft rejection. The transplant recipients, which had a previous history of grafting and/or presence of corneal neovascularization in more than 2 quadrants, were grouped into the high risk group. The others were grouped into a low risk group. All transplant recipients had completed at least 1-year of follow-up by April 1996. Seventy-nine transplant recipients were included in the antigen-matching analysis for HLA class I (HLA-A and -B), and for HLA-B matching. Eighty transplant recipients were analyzed to determine the effect of HLA-A matching. The definition of rejection-free graft survival and the management of postoperative immunosuppression therapy had previously been described in detail [12]. In brief: all transplant recipients were treated with local administration of steroids consisting of 0.1% dexamethasone eyedrops, three to five times a day, for 6 months. Then, the dexamethasone was changed to fluorometholone. Forty-four of 50 high-risk and 26 of 30 low-risk transplant recipients received systemic administration of steroids consisting of dexamethasone for either 3 days or tapering from 8 mg for 10-14 days. Many of the high-risk patients received cyclosporine treatment both topically and systemically. Nineteen transplant recipients were treated with systemic cyclosporine therapy, starting at 5 mg/kg and maintained at a level of 150 ng/ml for 3-6 months. The systemic cyclosporine recipients were also treated with topical cyclosporine; 0.05% cyclosporine eyedrops dissolved in a-cyclodextrin, five times a day, was used in 26 high-risk transplant recipients.

GENOMIC DNA EXTRACTION

The extraction of genomic DNA from corneal tissue samples was performed by the conventional phenol-chloroform method, according to the 11th International Histocompatibility Workshop protocol [16].

DNA TYPING FOR HLA CLASS I ALLELES

Low resolution DNA typing for HLA-A and HLA-B alleles were performed by the PCR-SSOP method [30-32].

PCR amplification:

Several oligonucleotide primers, which have been reported elsewhere, were used in the specific amplification of the HLA-A and HLA-B genes [30, 31]. In brief, 80 to 100 μl of reaction mixtures for each specific amplification were subjected to 30 cycles of PCR, in an automated oil bath PCR thermal sequencer (TSR-300, Iwaki Glass Co., Ltd, Chiba, Japan). The PCR conditions were different in each specific amplification according to the reference protocols. Fifty panel cell DNAs, isolated from healthy Japanese individuals, which had already been typed serologically, were used as controls in the HLA class I genotyping. PCR products were checked for amplification efficiency by 1% agarose and 10% acrylamide gel electrophoresis.

Dot blot hybridization:

PCR products were spotted onto a nylon

Table 1 Rejection episodes in study groups

Observed Study Group	Rejection Episode	No Rejection Episode	Total
High-Risk Group	17* (18)**	32	49 (50)
Low-Risk Group	6	24	30
Total	23	56	79 (80)

* - indicates the number of transplant recipients that were analyzed for HLA class I and HLA-B matching.
** - indicates the number of transplant recipients that were analyzed for HLA-A matching.

membrane (HybondTM-N+, Amersham International plc, Buckinghamshire, UK) and immobilized by alkaline denaturation in 0.4 N NaOH for 5 minutes followed by neutralization in 10×SSPE (1.5 M NaCl, 0.1 M Sodium Phosphate, 10 mM EDTA pH 7.4) for 10 minutes at room temperature. Membranes were prehybridized for 1 hour at 54℃ in 10 ml of hybridization buffer composed of 50 mM Tris-HCl (pH 8.0), 3.0 M tetramethylammonium chloride, 2 mM EDTA (pH 8.0), 5×Denhardt's solution, 0.1% SDS and 100 μg/ml heat denatured herring sperm DNA (Boehringer Mannheim GmbH, Germany). Then the membranes were hybridized with ^{32}P labelled SSOPs (by using γ^{32}P-ATP and T4 polynucleotide kinase) at 54℃ for 1 hour. A total of 107 SSOPs (77 for HLA-A, and 30 for HLA-B) were used for the hybridization. After hybridization the membranes were washed twice for 10 minutes each at room temperature in 2×SSPE with 0.1% SDS, and once for 30 minutes at 59℃ in TMAC solution (50 mM Tris-HCl, 3.0 M tetramethylammonium chloride, 2 mM EDTA and 0.1% SDS, pH 8.0). All steps were performed with constant gentle agitation. The membranes were washed in 2×SSPE for 2-3 minutes, then were exposed to an imaging plate for 1 hour at room temperature and were subjected to autoradiography to detect hybridization signals in a Bio-Image Analyzer BA100 (Fuji Photo Film Co., Ltd, Tokyo, Japan).

The assignment of HLA-A and HLA-B allele specificities was carried out by comparing hybridization patterns of all the probes used.

STATISTICS

The significance of differences between various groups was calculated by the chi-square test with Yate's correction, using raw data from HLA class I DNA typing. Allograft rejection-free survival curves were calculated using the Kaplan-Meier method. The statistical significance of differences in survival curves among groups was determined by the Cox-Mantel test.

RESULTS

The association between the HLA class I allele matching and a 1-year rejection-free graft survival was analyzed. The profile of observed transplant recipients in this study is shown in Table 1. During the 1-year follow-up period, 17 out of 49 (35%) high-risk transplant recipients and 6 out of 30 (20%) low-risk transplant recipients experienced a rejection episode. No significant difference in the occurrence of allograft rejection was seen between high- and low-risk groups.

Because recipients were selected randomly without previous HLA typing, and because donors (Caucasian) and recipients (Japanese) were from different racial background with a different allelic distribution of HLA class I genes, therefore as expected from previous data [12], 37 (47%) out of 79 transplant recipients in the study and 25 (51%) out of 49 high-risk transplant recipients had no matching for HLA class I (HLA-A and -B) alleles. Only 2 (3%) transplant recipients had a perfect four-allele matching at HLA-A and HLA-B.

First, we analyzed the effect of matching for HLA class I, HLA-A and HLA-B alleles together. Transplant recipients were grouped as follows: those with HLA class I matching, having one to four-alleles matched; and those without HLA class I matching, having no allele matched at HLA-A or HLA-B loci.

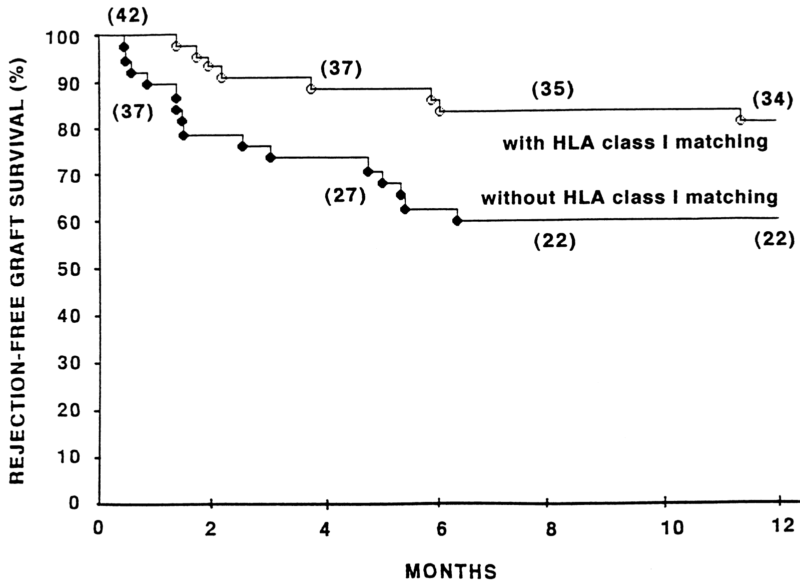

Fig. 1 Rejection-free graft survival in transplant recipients with HLA class I matching (one to four-alleles matched) as compared with transplant recipients without matching (no allele matched), in 79 transplant recipients (p=0.0258 by the Cox-Mantel test). Numbers in parentheses indicate rejection-free grafts under observation at the start of follow-up and every 4 months thereafter.

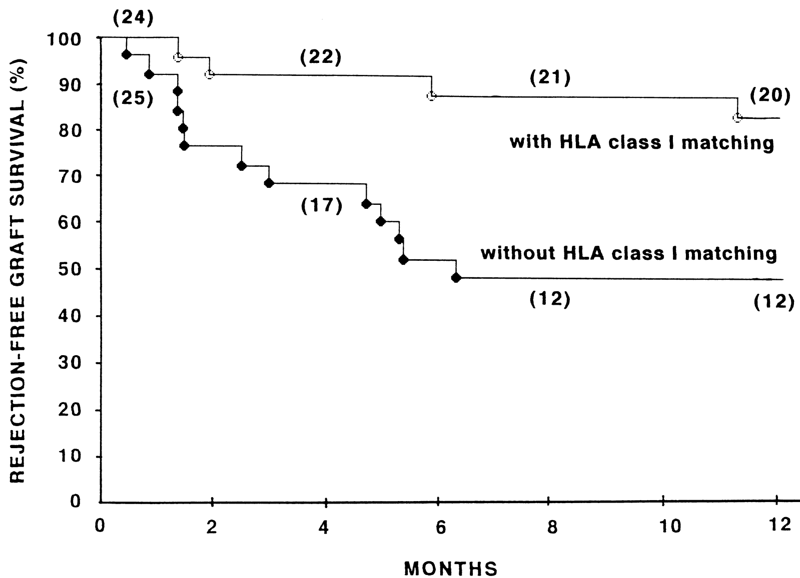

Fig. 2 Rejection-free graft survival of transplant recipients with matching at HLA class I (one to four-alleles matched) in comparison with those without matching (no allele matched), in 49 high-risk transplant recipients (p=0.0076 by the Cox-Mantel test). Numbers in parentheses indicate rejection-free grafts under observation at the start of follow-up and every 4 months thereafter.

As Figure 1 shows, the group with HLA class I matching had a significantly higher rate of rejection-free graft survival than without matching, in a total of 79 corneal transplant recipients, when high- and low-risk groups were analyzed together. The 1-year rejection-free graft survival of grafts with HLA class I matching was estimated at 81%, compared with 59% for grafts without matching (p=0.0258 by the Cox-Mantel test). Furthermore, there was also significant difference between two groups, in 49 high-risk transplant recipients (Figure 2). The 1-year rejection-free graft survival of grafts with HLA class I matching was estimated at 83%, compared with 48% for grafts without matching (p=0.0076 by the Cox-Mantel test).

Secondly, we examined matching for each, HLA-A, and HLA-B locus separately. Transplant recipients were grouped as follows: those with matching, having one or two-alleles matched; and those without matching, having no allele matched at each locus. A strong association between HLA-A matching and a rejection-free graft survival was observed in the total 80 corneal transplant recipients, when high- and low-risk groups were analyzed together (Figure 3). Transplant recipients with HLA-A matching had a rejection-free graft survival rate of 88%, as compared with 61% for those without HLA-A matching (p=0.0099 by the Cox-Mantel test). Then, we investigated the effect of HLA-A matching in 50 high-risk transplant recipients. As shown in Figure 4, the transplant recipients with HLA-A matching had a significantly higher rate of 1-year rejection-free graft survival (p=0.0154 by the Cox-Mantel test).

Next, we analyzed the effect of HLA-B matching. The Kaplan-Meier analysis of rejection rates showed no significant differences between the two groups in 79 transplant recipients, when high- and low-risk groups were analyzed together (p=0.5950 by the Cox-Mantel test, Figure 5). The same negative result was also observed in 49 high-risk transplant recipients (p=0.1741 by the Cox-Mantel test, Figure 6).

There were no significant differences between the two groups, in 30 low-risk transplant recipients (data not shown).

As we previously found that HLA-DPB1 matching had a beneficial effect, the effect of HLA-A matching was also analyzed in comparison with HLA-DPB1 matching, in order to clarify which one more strongly influences the outcome of corneal transplan-

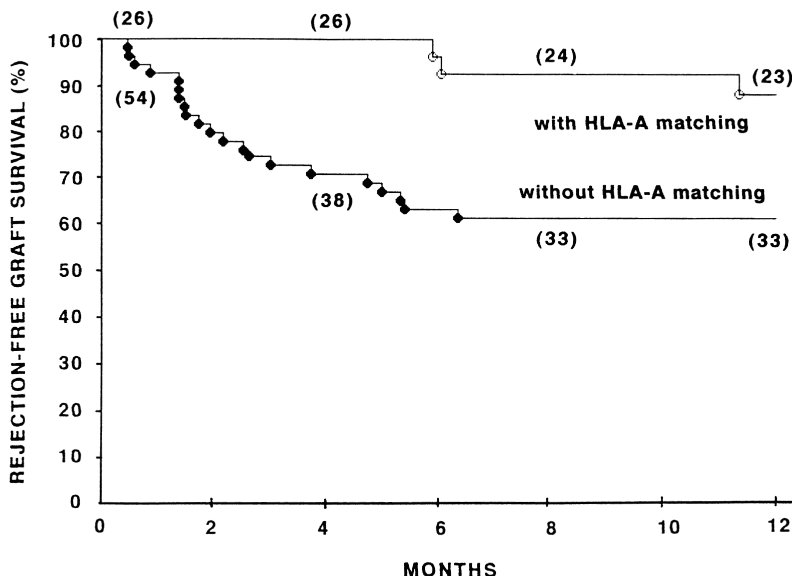

Fig. 3 Rejection-free graft survival of transplant recipients with HLA-A matching (one to two-alleles matched) in comparison with those without matching (no allele matched), in 80 transplant recipients (p=0.0099 by the Cox-Mantel test). Numbers in parentheses indicate rejection-free grafts under observation at the start of follow-up and every 4 months thereafter.

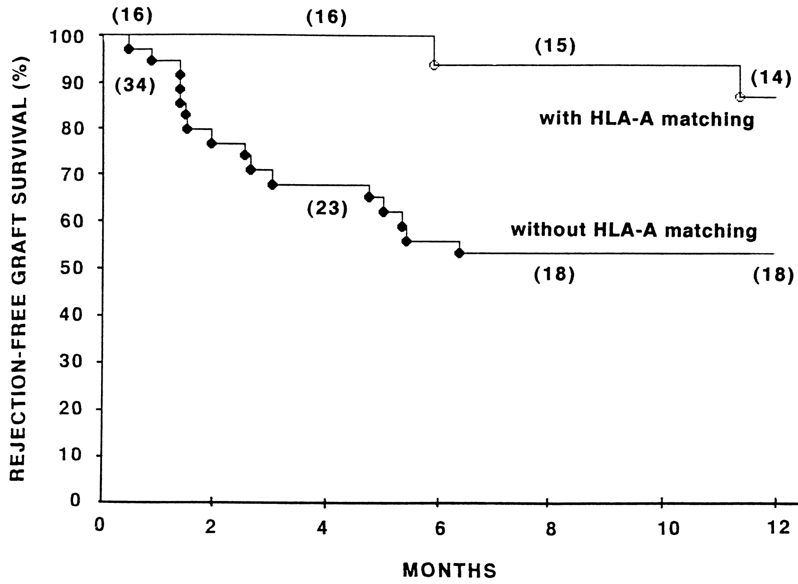

Fig. 4 Rejection-free graft survival in 50 high-risk transplant recipients with either matching at HLA-A (one to two-alleles matched) or without matching (no allele matched) (p=0.0154 by the Cox-Mantel test). Numbers in parentheses indicate rejection-free grafts under observation at the start of follow-up and every 4 months thereafter.

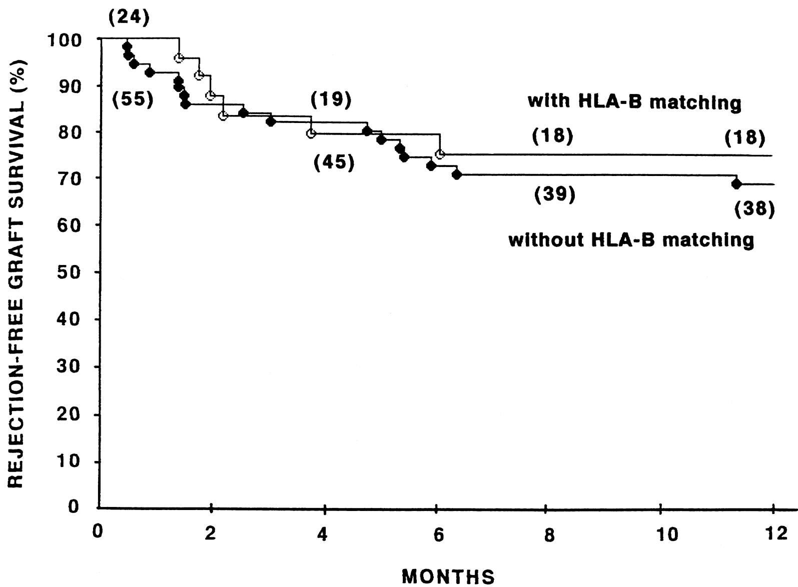

Fig. 5 Rejection-free graft survival of transplant recipients with HLA-B matching (one to two-alleles matched) in comparison with those without matching (no allele matched), in 79 transplant recipients (p=0.5950 by the Cox-Mantel test). Numbers in parentheses indicate rejection-free grafts under observation at the start of follow-up and every 4 months thereafter.

tation. However, neither matching for HLA-DPB1 nor HLA-A affected each other (data not shown).

Finally, the effect of matching for HLA-DPB1 or HLA-A was analyzed in short-term intervals of 3 months, within the 1-year follow-up. As shown in Table 2, in high-risk transplant recipients, matching for HLA-A was significantly effective 3 months after transplantation ($p<0.05$). In contrast, the HLA-DPB1 matching was significantly effective at 6, 9, and 12 months after transplantation.

DISCUSSION

A retrospective allele matching study for HLA class I at the DNA level was performed for the first time in corneal transplant recipients. The results demonstrated that, matching for HLA class I alleles, especially matching for HLA-A alleles alone, were significantly beneficial. First, transplant recipients, who had at least one-allele matching (or more) for HLA class I, had a significantly higher rejection-free graft survival rate compared with those without matching. Secondly, the analysis of matching at HLA-A and -B loci separately, showed that matching

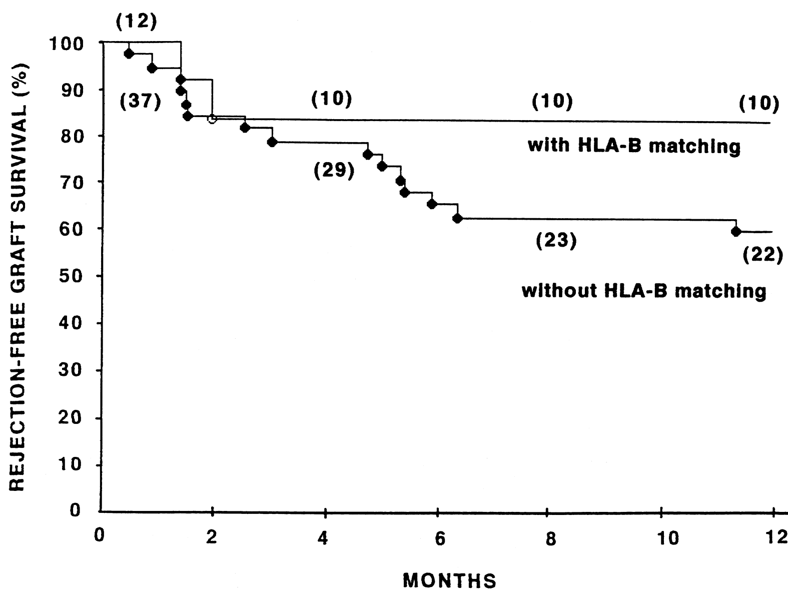

Fig. 6 Rejection-free graft survival in 49 high-risk transplant recipients with either matching at HLA-B (one to two-alleles matched) or without matching (no allele matched) ($p=0.1741$ by the Cox-Mantel test). Numbers in parentheses indicate rejection-free grafts under observation at the start of follow-up and every 4 months thereafter.

Table 2 Comparison of effects of HLA-DPB1 and HLA-A matching in high-risk corneal transplant recipients in a defined periods of 1-year follow-up

Matching	3 months RR*	p**	6 months RR	p	9 months RR	p	12 months RR	p
HLA-DPB1	11.07	0.076	19.96	0.013	24.75	0.006	7.68	0.024
HLA-A	14.14	0.041	26.24	0.004	9.22	0.012	5.17	0.039

(Follow-up months)

* RR - relative risks, calculated by chi-square test
** p - p-values, calculated by chi-square test

at HLA-A was strongly associated with a higher rate of 1-year rejection-free graft survival. However, we did not find an independent effect of HLA-B matching. These findings, therefore, confirmed the observations by other investigators [2-5, 8, 9].

In agreement with the findings of our previous study for HLA class II allele matching, we found no beneficial effect of HLA class I matching in the low-risk group. Perhaps, this noneffectiveness of HLA matching in low-risk transplants might be partially explained by the restricted low level of HLA expression in unvascularized, uninflamed, native corneal tissue.

Moreover, because the study was retrospective, we have taken into consideration the possibility that immunosuppressive therapy could have unwittingly affected the graft outcome. Although more intensive immunosuppression, including topical and systemic cyclosporine, were given more in the high-risk patients, we did not find a significant difference in the immunosuppressive therapy between recipients who received HLA-A matched and unmatched corneas (data not shown). In addition, numerous other critical variables such as presence of a primary disease in the recipients, HLA distribution within the host and donor population and the possible impact of a particular donor or recipient allele rather than the level of match, were not different between the groups (data not shown).

When the effect of HLA class II matching was investigated, we found that HLA-DPB1 matching was significantly associated with a higher rate of 1-year rejection-free graft survival in high-risk transplant recipients [12]. The present study for HLA class I matching indicated the impact of HLA-A matching. Unfortunately, it was impossible to clarify which, HLA-DPB1 or HLA-A, had more strongly influenced the outcome of corneal grafts in our small scaled study. On the other hand, it should be pointed out that, the low resolution PCR-SSOP method was used in HLA class I typing.

The effect of various risk factors may appear during the clinical course after transplantation. Our analysis of matching for HLA-DPB1 or HLA-A, in short-term intervals of 3 months within the 1-year follow-up, revealed that the significance of HLA-A matching appears earlier, i.e. at 3 months after transplantation (p=0.041). Whereas, the HLA-DPB1 matching becomes significantly effective for the first time at 6 months after transplantation (p=0.013). It therefore, was reasonable to propose a hypothesis that matching for HLA-A alleles might be closely associated with allograft rejection in the early post-transplantation period, and matching for HLA-DPB1 alleles might be responsible for later allograft rejection. Thus, large numbers of cases and longer follow-up are certainly required in order to clarify any independent effect of HLA-DPB1 or HLA-A matching in corneal transplantation and also which one is the most important for short or long-term rejection-free graft survival.

In conclusion, the present study indicates that HLA-A matching in corneal transplantation represents a significant independent effect on allograft rejection and suggests that, when possible, corneal transplant recipients should receive allografts from donors with whom they are matched for at least one HLA-A allele. Subsequently, large scale application of molecular typing techniques and the prospective typing in high-risk patients should be seriously considered.

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