Histocompatibility Y antigen compatibility and allograft rejection in corneal transplantation

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Abstract

Purpose **To assess the effects of histocompatibility Y (H-Y) antigen matching on the rate of corneal allograft rejection after penetrating keratoplasty (PKP).**
Methods **We retrospectively investigated the graft survival rate and rejection-free graft survival rate after PKP in 396 eyes. The compatible combinations of H-Y antigen included male donors and male recipients (*n* = 135), female donors and male recipients (*n* = 107), and female donors and female recipients (*n* = 60). Incompatible combination was from male donors and female recipients (*n* = 94). The eyes were classified into two groups – high-risk (168 eyes) and low-risk (228 eyes) – depending on the degree of vascularisation in the recipient corneas or a history of previous allograft rejection. Data were analysed using the Kaplan–Meier life table method, the log-rank test and the Cox proportional hazards model.**
Results **In both the high-risk and low-risk groups, the graft survival and rejection-free graft survival rates were not affected by the H-Y compatibility. The graft survival (*p* < 0.001) and rejection-free graft survival (*p* < 0.001) rates were higher in the low-risk group than in the high-risk group. High-risk PKP was associated with greater risk of graft failure (risk ratio, 2.33) and rejection (risk ratio, 2.05) than low-risk PKP.**
Conclusion **H-Y antigen matching does not influence the rate of allograft rejection after PKP.**

Key words Compatibility, Graft survival rate, H-Y antigen, Penetrating keratoplasty, Rejection-free graft survival rate

Because blood vessels and lymphatics are absent in the normal cornea, the risk of allograft rejection after penetrating keratoplasty (PKP) is assumed to be lower than that after other organ transplantations. However, Allograft rejection does occur in about 20–30% cases after PKP.[1–3]

Rejection is triggered when the host lymphocytes recognise the major and minor histocompatibility antigens expressed by cells of the corneal grafts. In humans, the effects of matching of the major histocompatibility complex (MHC) in corneal transplantation are subject to controversy.[3–9] Some previous studies have reported that the matching of HLA- and A-B antigens decreases the rate of graft rejection,[3–7] whereas others reported that the matching of HLA-A, -B and -DR antigens does not decrease the rate of allograft rejection.[8,9]

Histocompatibility Y (H-Y) antigen is a male-specific minor histocompatibility antigen and was originally found as a transplantation antigen by Eichwald and Slimser in 1955.[10] They found that skin grafts from male donors could be rejected by female recipients from certain strains of inbred mice.[10] The term H-Y antigen was introduced by Billingham and Silvers in 1960, who reported that the male-specific antigen could function as a classical transplantation antigen responsible for homograft rejection.[11] H-Y antigen is located on the long arm of chromosome Y,[12] but its molecular nature is not known. H-Y antigen has been shown to play an important role in graft rejection and graft-versus-host disease in various tissues following transplantation of a male tissue to females.[13–17] A mismatch of H-Y antigen in heart transplantation significantly increased the number of rejection episodes.[13] The importance of H-Y compatibility has been demonstrated in human bone marrow[14,15] and liver transplantation.[16] The effect of H-Y compatibility on renal transplant rejection responses, however, remains controversial.[15,17–19]

The functional expression of H-Y antigen in human corneal tissue was demonstrated by Goulmy *et al.*[20] The effects of H-Y antigen compatibility on the outcome of PKP have been reported only in mice.[21,22] Ray-Keil and Chandler,[21] using the PKP model in mice, reported that the rejection rates of H-Y antigen-compatible transplants (5%) were lower than those of H-Y antigen incompatible transplants

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(36%). In contrast, He et al.[22] observed no rejection of male corneal grafts by naive female C57BL/6 mice for more than 100 days after transplantation. There have been no reports of the influences of H-Y antigen compatibility in human corneal transplantation.

In this study we assessed the influence of H-Y antigen compatibility on the corneal allograft survival in 396 donor–recipient pairs using Kaplan–Meier life table analysis,[23] the log-rank test and the Cox proportional hazards·model.[24]

Materials and methods

Among the 429 consecutive PKPs performed between October 1987 and September 1997 at the University of Tokyo Hospital, 396 eyes of 334 patients who were followed up for at least 6 months after surgery were included in the current retrospective study. We did not match HLA antigens between donors and recipients. The grafts were all allocated randomly regardless of HLA antigens and sex. The compatible combinations of H-Y antigen for the donors and recipients were male donors and male recipients (n = 135), female donors and male recipients (n = 107), and female donors and female recipients (n = 60). Incompatible combination was male donors and female recipients (n = 94). The corneal transplantations were classified into two groups (high-risk and low-risk) and the effect of H-Y antigen compatibility was examined in each group. The high-risk transplantation group involved recipients who had corneal vascularisation in two or more quadrants of the cornea pre-operatively or who had a history of graft failure. One hundred and sixty-eight PKPs were performed on the high-risk recipients, of which 127 were H-Y antigen-compatible and 41 H-Y antigen-incompatible. Two hundred and twenty-eight PKPs were performed on the low-risk recipients, 175 of which were H-Y antigen-compatible and 53 H-Y antigen-incompatible.

The pre-operative diagnoses are shown in Table 1. The pre-operative diagnoses were similar between the H-Y antigen-compatible and H-Y antigen-incompatible subgroups. The pre-operative diagnoses in the high-risk group included transplant rejection (39.9%) and non-herpetic keratitis (19.0%). Diagnoses in the low-risk group mainly consisted of keratoconus (35.1%) and bullous keratopathy (29.8%). The mean post-operative follow-up period was 49 ± 31 months (mean ± SD) in the high-risk group (range 6–123 months) and 45 ± 30 months in the low-risk group (range 6–122 months).

A corneal graft rejection was defined as development of corneal oedema and immunological signs, such as a rejection line, infiltrative keratic precipitates and anterior segment inflammation.[25] The diagnosis of rejection was made only if the transplant had remained clear for at least 2 weeks after surgery. The clarity of the corneal graft was determined clinically using a slit-lamp biomicroscope.

Donor eyes, enucleated aseptically, were maintained in preservation medium (EP-II, Kaken Pharmaceuticals, Osaka, Japan) at 4 °C. Transplantations were done within 72 h after enucleation. Sclerocorneal rims were made at the time of transplantation, and the donor buttons were punched from the endothelial side using Weck's disposable trephines. The mean diameter of the corneal graft was 7.70 ± 0.60 mm (range 5.0–12.0 mm). The diameter of the corneal graft was 0.25–0.50 mm larger than that of the recipient cornea. The grafts were sutured to the recipient corneas with eight interrupted 10-0 nylon preplaced sutures followed by running or interrupted 10-0 nylon sutures. Lensectomy, anterior vitrectomy, intraocular lens implantation or intraocular lens removal were performed as necessary. The patients received a subconjunctival injection of dexamethasone (2 mg) and ofloxacin ointment at the end of surgery. Systemic prednisolone (30–60 mg/day) and topical betamethasone (1 mg/ml), ofloxacin and tropicamide were prescribed as post-operative treatment. Topical treatment was tapered off over several months: from 6 times a day post-operatively to 4 times a day at 3 months, 3 times daily at 6 months, and twice daily at 12 months. The corneal sutures were usually removed 12–18 months after surgery.

Table 1. *Pre-operative diagnoses*

	HY-compatible	HY-incompatible	Overall
High-risk transplants (n = 168)			
Transplant rejection	55 (43.3%)	12 (29.3%)	67 (39.9%)
Non-herpetic keratitis	23 (18.1%)	9 (22.0%)	32 (19.0%)
Bullous keratopathy	20 (15.7%)	7 (17.1%)	27 (16.1%)
Herpes keratitis	17 (13.4%)	6 (14.6%)	23 (13.7%)
Corneal dystrophies and degenerations	5 (3.9%)	6 (14.6%)	11 (6.5%)
Chemical burns	6 (4.7%)	0	6 (3.6%)
Keratoconus	1 (0.8%)	1 (2.4%)	2 (1.2%)
Low-risk transplants (n = 228)			
Keratoconus	68 (38.8%)	12 (22.6%)	80 (35.1%)
Bullous keratopathy	53 (30.3%)	15 (28.3%)	68 (29.8%)
Non-herpetic keratitis	26 (14.9%)	14 (26.4%)	40 (17.5%)
Herpes keratitis	14 (8.0%)	9 (17.0%)	23 (10.1%)
Corneal dystrophies and degenerations	12 (6.9%)	3 (5.7%)	15 (6.6%)
Chemical burns	2 (1.1%)	0	2 (0.9%)

Patients who developed immunological rejection were treated intensively with topical and systemic steroids. The standard treatment for allograft rejection includes the installation of steroid eye drops every 1 or 2 h, subconjunctival injection of 1.2–2.0 mg dexamethasone, and systemic administration of prednisolone (30–60 mg/day) for 1–2 weeks with tapering off.

The graphical graft survival curves and rejection-free graft survival curves were obtained by the Kaplan–Meier method,[23] and the data were analysed using the log-rank test. The Cox proportional hazards model[24] was employed to evaluate two variables as possible contributory factors to the occurrence of graft failure or rejection. The tested variables were H-Y antigen incompatibility and recipient bearing risk (two categories of high risk and low risk as previously defined).

Results

In the high-risk group, graft survival (Fig. 1, $p = 0.23$) and rejection-free graft survival (Fig. 2, $p = 0.70$) rates were not significantly different between the H-Y antigen-compatible and H-Y antigen-incompatible subgroups. In the low-risk group there was no significant difference between the H-Y antigen-compatible and H-Y antigen-incompatible subgroups as regards graft survival (Fig. 3, $p = 0.70$) and rejection-free graft survival (Fig. 4, $p = 0.71$) rates. Graft survival (Fig. 5, $p < 0.001$) and rejection-free graft survival (Fig. 6, $p < 0.001$) rates were significantly higher in the low-risk group than in the high-risk group.

H-Y antigen incompatibility was associated with approximately the same degree of excess risk of graft failure (risk ratio, 0.75; $p = 0.23$) and rejection (risk ratio, 0.99; $p = 0.97$) as H-Y antigen compatibility (Table 2). High-risk PKP, however, was associated with greater
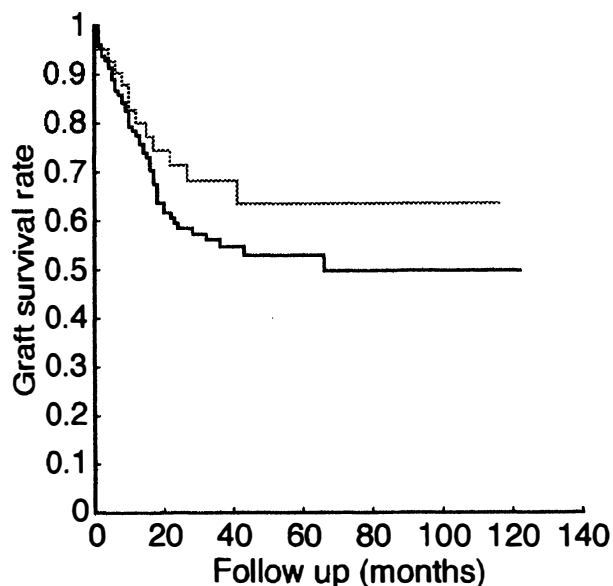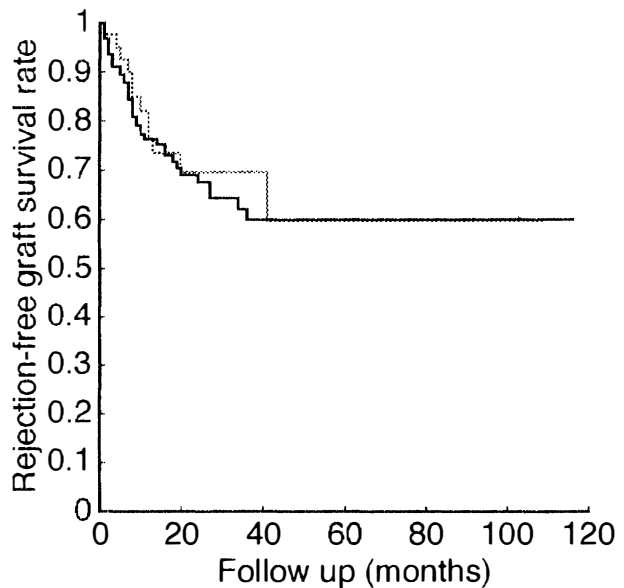
Fig. 2. *Rejection-free graft survival in the H-Y antigen-compatible (continuous line) and H-Y antigen-incompatible (broken line) high-risk subgroups estimated by the Kaplan–Meier method. Log rank test:* $\chi^2 = 0.15$, $p = 0.70$.

risk of graft failure (risk ratio, 2.33; $p < 0.0001$) and rejection (risk ratio, 2.05; $p = 0.0006$) than low-risk PKP (Table 2).

Discussion

In humans, the overall number and complexity of minor histocompatibility antigens remains uncertain. The ABO and H-Y antigens are known as human minor histocompatibility antigens. Several studies have reported that ABO matching significantly decreases the rate of corneal graft rejection in high-risk recipients,[2,9] but others found no significant effect of ABO matching on corneal graft survival.[3,5,26–28]
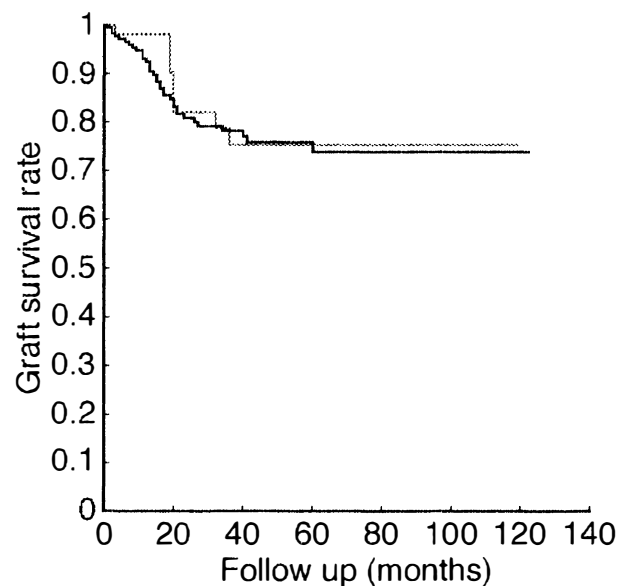
Fig. 1. *Graft survival in the H-Y antigen-compatible (continuous line) and H-Y antigen-incompatible (broken line) high-risk subgroups estimated by the Kaplan–Meier method. Log-rank test:* $\chi^2 = 1.43$, $p = 0.23$.

Fig. 3. *Graft survival in the H-Y antigen-compatible (continuous line) and H-Y antigen-incompatible (broken line) low-risk subgroups estimated by the Kaplan–Meier method. Log rank test:* $\chi^2 = 0.14$, $p = 0.70$.
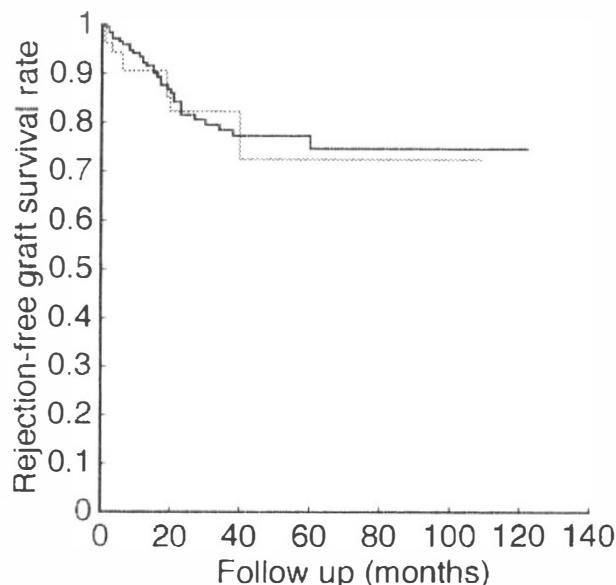
Fig. 4. *Rejection-free graft survival in the H-Y antigen-compatible (continuous line) and H-Y antigen-incompatible (broken line) high-risk subgroups estimated by the Kaplan–Meier method. Log rank test:* $\chi^2 = 0.14$, $p = 0.71$.
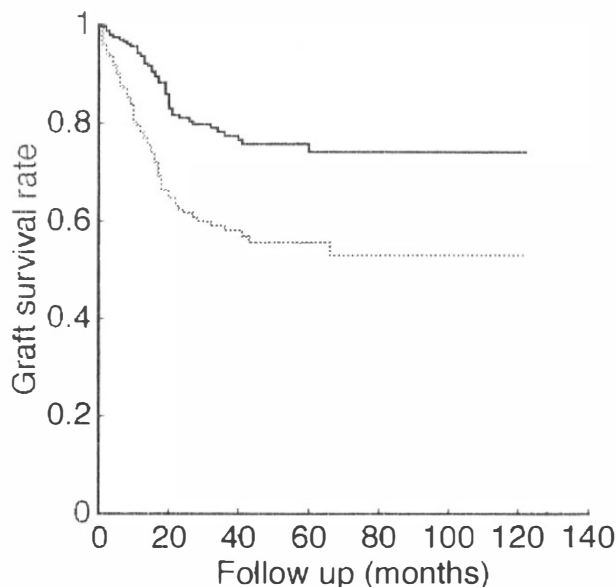

Fig. 5. *Graft survival in low-risk (continuous line) and high-risk (broken line) groups estimated by the Kaplan–Meier method. Log-rank test:* χ^2 *19.8, $p = 0.0001$.*

The influences of H-Y antigen compatibility in human corneal transplantation have not been reported; however, those of sex differences were reported.[10,28–30] Cherry *et al.*[29] analysed 350 corneal transplants and found no significant effect of sex compatibility on the overall corneal graft clarity. Volker-Dieben *et al.*[30] analysed 432 transplants and found a significant influence of sex difference between the donors and recipients,[28] but in a
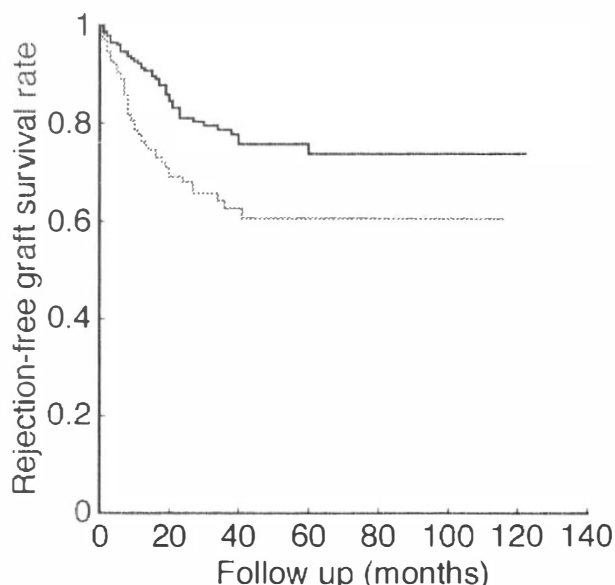

Fig. 6. *Rejection-free graft survival in low-risk (continuous line) and high-risk (broken line) groups estimated by the Kaplan–Meier method. Log-rank test:* $\chi^2 = 12.3$, $p = 0.0004$.

later analysis of 1218 transplants they found no influence of donor–recipient gender matching on the graft failure rate.[30] Sanfilippo *et al.*[4] analysed 97 transplants in high-risk recipients and found no significant effect of sex compatibility on the incidence of corneal rejection reactions.

Volker-Dieben *et al.*[30] found that a female donor cornea afforded significantly better graft survival at 1 year in female (85%) than in male (76%) recipients. A male donor did not affect graft survival in male recipients (79%) compared with female recipients (80%). They did not mention the mechanism and reason for their results, and we also have no clear explanation of their data. In the current study, the combination of male donor–male recipient did not improve the graft survival rate compared with the male donor–female recipient combination. Moreover, the female donor–male recipient combination did not offer better graft survival rate than the female donor–female recipient combination. We could not find any influences of sex differences between the donors and recipients.

We did not match HLA antigens between donors and recipients. The grafts were all allocated randomly regardless of HLA antigens and sex. The matching of HLA antigens would be effective in preventing allograft rejection after PKP, especially in high-risk patients, but it is very difficult to find a donor and recipient whose HLA antigens match, because the HLA antigens of donors are not usually examined in Japan. However, it could be argued that H-Y incompatibility is only likely to be

Table 2. *Adjusted relative risk of graft failure and rejection for penetrating keratoplasty*

Risk factor	Graft failure		Rejection	
	Relative risk	p	Relative risk	p
H-Y antigen-incompatible	0.75	0.23	0.99	0.97
High-risk	2.33	< 0.0001	2.05	0.0006

important in HLA-DR matched grafts and that not matching HLA antigens may contribute to the failure of the current study to show an influence of H-Y incompatibility. Further studies are necessary to elucidate the relationship between HLA antigens and H-Y antigen in corneal allograft rejection.

Thus, we conclude that the H-Y antigen does not contribute significantly to the mechanism and development of graft rejection in allograft PKP examined in this study.

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