A prospective study of histocompatibility testing for keratoplasty in high-risk patients

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SUMMARY Forty high-risk patients awaiting corneal grafting were randomly selected from a 7-year cumulative list. Twenty were grafted with HLA-A and HLA-B antigen matched material. The other 20 patients were used as controls. Graft survival at two years was 60% (12/20) in the control group and 90% (18/20) in the study group; at five years it was 55% (11/20) in the control group and 85% (17/20) in the study group.

In recent years corneal graft operations have become routine in the treatment of blindness caused by corneal trauma, oedema, inflammation, dystrophy, and congenital abnormalities that can result in opacification of the cornea. However, a significant percentage of grafts fail weeks to months after surgery. These are generally believed to be caused by immunomediated allograft rejection.¹

Several studies have shown that the rate of corneal graft rejection increases according to the degree of vascularisation of the cornea.¹⁻³ Several retrospective studies have suggested that increased matching between HLA-A and HLA-B locus antigens yields improved corneal allograft survival,^{4,6} but some other studies have failed to confirm these findings.

This study gives the results after five years in 20 high-risk patients who had maximised HLA-A and HLA-B antigen matching and 20 high-risk control patients.

Patients and methods

This prospective study was started in Ankara University School of Medicine Eye Department between April 1978 and September 1979. Forty patients randomly chosen from the waiting list for a keratoplasty operation were identified as being at high risk of graft rejection by virtue of having significant vascularisation of one or more quadrants of corneal stroma or having had prior irreversible corneal allograft rejection.

These patients were divided into subgroups,

namely severe vascularisation involving four quadrants of the cornea, medium vascularisation involving two to three quadrants, and mild vascularisation of only one quadrant (Table 1). Thirty were male and 10 were female, and their ages were between 7 and 70 years.

All the patients had less than 0.1 (6/60) (Snellen) visual acuity before operation. They all had normal intraocular pressure, and none had ever had a blood transfusion, nor were they known to be sensitised with any material. But one patient had had a previous corneal allograft rejection.

The patients were tested before transplantation to identify their HLA-A and HLA-B genotype. Blood was taken from the donor's jugular vein 30-60 min after death; at the same time donor corneas were taken for the surgery.

HISTOCOMPATIBILITY TESTING

The standard microlymphocytotoxicity test was used for HLA serological typing of donors and recipients. We have used National Institute of Allergy and Infectious Disease Serum Bank material for the confirmation of antigen assignment. For the recognition of sera 14 HLA-A and 15 HLA-B specificities were used. The percentage of reactive antibody reflected the percentage of panel cells showing at

Table 1 Corneal vascularisation 20 controls and 20 cross matched patients

	Severe	Medium	Mild
Cross-matched	8	8	4
Controls	8	7	5

least 20% lysis by the patient's serum regardless of the method used.⁷

PRESERVATION OF CORNEAS AND TREATMENT

Donor corneas for patients in both study and prospective control groups were stored in McCarey Kaufman medium until keratoplasty. Surgery was performed within 50 hours of the donor's death. All donor buttons were punched from the posterior corneal surface on to a Teflon block with a trephine 0.5 mm larger than that used to incise the recipient bed. The standard surgical technique for penetrating keratoplasty was employed for all patients, with the use of interrupted 10/0 nylon sutures, approximately four sutures per quadrant.

After the operation topical steroids were applied two or four times a day for the first four weeks and then tapered over another four-week period. Most patients were maintained on one drop of topical steroid every day.

The sutures were removed when they loosened or if visible vessels encroached on the wound edge and began to migrate round the graft margin. Graft rejections were treated by hourly topical steroids and ointment once each evening. When severe inflammation was present, a periocular injection of 10 to 20 mg soluble dexamethasone was also given. Systemic steroids were withheld unless an extremely severe reaction was detected.

Results

The risk factors for all patients included in the study and in the retrospective control groups are listed in Table 2. No significant differences in age, sex, primary corneal disease, or vascularisation were seen between the groups. Most rejections occurred within nine months of transplantation in both groups.

The overall graft survival was 60% (12/20) for the control group and 90% (18/20) for the cross-matched group, with mean follow-up times of 24 months. The difference in survival was statistically significant (Fisher's exact test, $p < 0.03$).

The effect of HLA matching was examined for patients in the cross-matched group (Table 3). All rejections occurred in patients receiving poorly matched donor grafts. In the cross-matched patients of the severe vascularisation group only one rejected.

There was also one rejection from those who had medium vascularisation. There was a statistical difference in survival between the control group and the cross-matched group for severe and medium vascularisation, but no difference for the mild vascularisation groups (Table 4).

The effect of HLA antigen matching and preformed antibodies was examined for patients in the study and control group. Of the 20 patients in the study group 19 had a two locus HLA-A, HLA-B antigen match, and only one patient had a three locus antigen match. The patient who had a three locus antigen match had a severely vascularised leucoma. The vision was counting fingers before the operation. The graft was clear and vision restored to 0.4 after the operation. Only three patients had graft rejection in this study group (85%) (Table 5).

Of the 20 patients in the control group eight had had no antigen matching and 12 patients had had only one locus matching. The graft survived in five of those eight patients and in six of the 12. There was no

Table 2 Causes of corneal opacification

	Total	Control	Cases
Postinflammatory simple leucoma	27	14	13
Postinflammatory anterior synechiae	4	2	2
Viral infection	5	3	2
Burns	2	1	1
Previous graft	1	—	1
Corneal dystrophy	1	—	1

Table 3 Graft survival for 24 months

	Clear graft	Rejection	Total
Cross-matched	18 (90%)	2 (10%)	20
Controls	12 (60%)	8 (40%)	20

Table 4 Graft survival rate according to the vascularisation in 24 months

	Severe		Medium		Mild	
	Clear	Reject	Clear	Reject	Clear	Reject
Cross-matched	7	1	7	1	4	—
Controls	5	3	3	4	4	1

Table 5 HLA-A and HLA-B antigens

	No match		One locus match		Two locus match		Three locus match		Total
	Clear	Reject	Clear	Reject	Clear	Reject	Clear	Reject	
Cross-match	—	—	—	—	16	3	1	—	20
Controls	5	3	6	6	—	—	—	—	20

Table 6 Graft survival after five years

	Clear graft	Rejection	Total
Cross-matched	17 (85%)	3 (15%)	20
Controls	11 (55%)	9 (45%)	20

Table 7 Graft survival rate related to the preoperative vascularisation of the cornea: five years follow-up

	Severe		Medium		Mild	
	Clear	Reject	Clear	Reject	Clear	Reject
Cross-matched	6	2	7	1	4	—
Controls	4	4	3	4	4	1

statistically significant difference between these groups.

The overall survival of the graft at five years was 55% (11/20) for the control group and 85% (17/20) for the cross-matched group. The difference in survival was statistically significant (Fisher's exact test, $p < 0.05$).

The effect of HLA matching was examined for patients in the cross-matched group. All rejections occurred in patients receiving poorly matched donor grafts (Table 6). One further patient who had been cross-matched and who had severe vascularisation rejected his graft five years later. There was no difference between the medium and mild vascularisation groups at five years (Table 7).

Discussion

The results of this study indicate that donor corneal selection based on histocompatibility gives rise to a significant improvement in graft survival in patients who are at high risk of allograft rejection. Several recent studies have examined the role of HLA antigen compatibility in high-risk corneal transplant patients. Most of them include only a few cases and lack control groups. The difference in the overall graft survival between our control series (60%) and the previously reported 30–40%¹⁴ may be explained by improved surgical technique and follow-up. However, better donor selection provides a significant increase in graft survival for our cross-matched group, which was 90% in the 24 months mean follow-up period.

Transplantation of the avascular cornea differs significantly from that of a vascular graft, such as the kidney, but when the recipient cornea is vascularised the cornea may provide potential immune sensitivity. For this reason the allograft rejection rate is increased. This shows that the cornea has specific HLA

antigens just as other tissues have.⁸ The question of blood group antigens in the cornea remains controversial. The effect of blood group compatibility on corneal graft survival is unclear and probably minimal, even though blood group compatibility has been shown to affect renal transplantation.^{9,13}

This is the first prospective study of HLA typing in cases of corneal graft rejection among high-risk patients to be reported. All the retrospective studies also support the role of histocompatibility in patients at high risk of graft rejection, and, as reported by Ehler and Kissmeyer-Nielsen,¹⁴ the success rate is high. Another similar study by Foulks *et al.*¹⁵ showed a 95% rate for the study groups and 52% for control groups.

In this study graft survival was 55% (11/20) for the control group and 85% (17/20) for the cross-matched group at five years. Only one cross-matched patient with severe vascularisation rejected within this time. And in the control group the cases of graft rejection increased from eight to nine in the five years. Most of these rejections were in patients who had severe and medium vascularisation.

The extended preservation time obtained by the storage of the corneas in M–K media makes pre-operative testing possible. The increasing costs are at an acceptable level in an established transplantation testing laboratory.

According to this study higher graft survival rates, especially in high-risk patients, have resulted from the use of HLA-A and HLA-B matching for both the recipients and the donors.

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