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An adverse matching effect for the HLA-B locus in corneal transplantation

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Abstract The results of tissue typing on 115 recipient/donor pairs prior to corneal grafting were analyzed with the proportional hazard regression model for the incidence of the first rejection episode and for graft failure from rejection. Like other investigators, we found that a previously failed corneal graft and the degree of recipient corneal vascularization were significant risk factors for graft rejection. ABO blood group matching had no effect. The absence of mismatches in both the HLA-A and HLA-DR loci decreased the incidence of rejection. However, no difference was observed for the presence of one versus two mismatches. Regression results for the HLA-A and DR loci were not significant. Surprisingly, matching for one or both HLA-B alleles resulted in a significantly higher incidence of graft rejection episodes ($P < 0.005$) and of graft failure ($P < 0.052$). This adverse matching effect for the B locus was proportional to the number of mismatches.

Key words Corneal transplantation, HLA-B locus · HLA-B locus, corneal transplantation

Introduction

In general, corneal transplantation has a higher success rate than transplantation of other organs, but it is recognized that vascularization of the recipient cornea bed or a previously failed graft can reduce graft survival to 35 %–65 % [1, 10, 15, 20, 21, 23]. A number of early studies show a beneficial effect of HLA-A and HLA-B matching, while conflicting results are reported for matching at the DR locus [1, 17, 19, 20]. Three large studies that yielded controversial results have recently been conducted. A single-center study in the Netherlands showed a clear improvement in graft survival for matching the HLA-A, -B, and -DR loci [5]. A multicenter study conducted in the United Kingdom also showed better results after matching for the HLA-A and -B loci. However, the authors reported substantially decreased graft survival in patients matched at the HLA-DR locus [19]. In the Collaborative Corneal Transplantation Studies, a multicenter study conducted in the United States found a beneficial effect of ABO matching, but did not find any statistical benefit from HLA-A, -B, or -DR matching [4].

This paper reports a small study on the effect of HLA matching in a cosmopolitan society and discusses the role of the immune response to viral agents in transplantation.

Patients, materials, and methods

We tissue typed 115 patients undergoing penetrating keratoplasty who received grafts from tissue-typed cadaver donors. We used the standard microlymphocytotoxicity test for tissue typing [16]. The majority of patients belonged to the Cape Coloured mixed ancestry group, which is not very stratified; approximately one-third was either black or white. The donors came from the same populations. We did no ethnic matching. Patients and donors were tissue typed and matched for the HLA-A, -B and -DR alleles. The HLA allele frequencies in our populations have been described previously [6]. The number of previous grafts in the operated eye and

the degree of recipient corneal vascularization were noted. The degree of vascularization was assessed by counting the number of vessels crossing the proposed recipient/donor junction and by the number of corneal quadrants of vascularization [8]. Nine patients received grafts for documented herpes simplex virus (HSV) corneal disease. The group included 50 high-risk cases. Two recipients developed recurrent HSV infections, but neither rejected the graft. Four patients who rejected the graft due to nonimmunological causes were excluded from the study group.

The keratoplasty technique was identical for all patients. Most donor grafts were between 7.5 and 8.5 mm in diameter and were 0.1–0.5 mm larger than the recipient bed. Donor corneas were preserved as whole eyes, with the corneoscleral disc being removed just prior to grafting, or as discs preserved in culture medium [McCarey-Kaufman solution (M.K.) or KSol solution] at 4 °C. A number of patients had simultaneous extracapsular cataract extraction, in some later cases with placement of posterior chamber intraocular lenses. A viscoelastic substance was used to protect donor endothelium and to dissect any iris adhesions. Sixteen buried, interrupted 10/0 nylon sutures or a continuous 10/0 nylon suture was used to secure the graft. The anterior chamber was reformed with balanced salt solution at the completion of surgery, and subconjunctival injections of gentamicin, 20 mg, and betamethasone (Celestone), 2 mg, were given. Postoperatively, all patients initially received topical dexamethasone 0.1 % or prednisolone 1 % drops four times daily. The frequency was reduced over the ensuing months and was stopped at 6 months unless persistent inflammation required extended therapy. Topical antibiotics and cycloplegics were given when necessary in the early postoperative period.

Clinical follow-up ranged from 15 to 104 months. We analyzed the results with the Cox proportional hazard regression model with the time to the first rejection episode; the incidence of the reaction was the censoring factor. The same procedure was followed for graft failure from rejection. For the purpose of analysis, patients were regarded as having either no previous graft or more than one. With corneal vascularization, patients were categorized as having 0, 1, or ≥ 2 quadrants of vascularization. ABO blood groups were analyzed for compatibility or incompatibility. HLA-A, -B, and -DR loci were each analyzed for 0, 1, or 2 mismatches.

Since the groups with no mismatches for the various HLA loci were too small to calculate Kaplan-Meier plots, survival curves were constructed by calculating the cumulative percentages of graft survival and of rejection-free survival. This was done for 99 patients with a follow-up period of at least 30 months.

Table 1 *P* values for the Cox proportional hazard regression model in relation to incidence of first graft reaction and graft failure

Variable	Graft reaction *P* value	Graft failure *P* value
Previous graft	0.009	0.016
Vascularization	< 0.001	0.005
ABO	0.932	0.434
HLA-A	0.562	0.635
HLA-B	0.005	0.052
HLA-DR	0.311	0.413

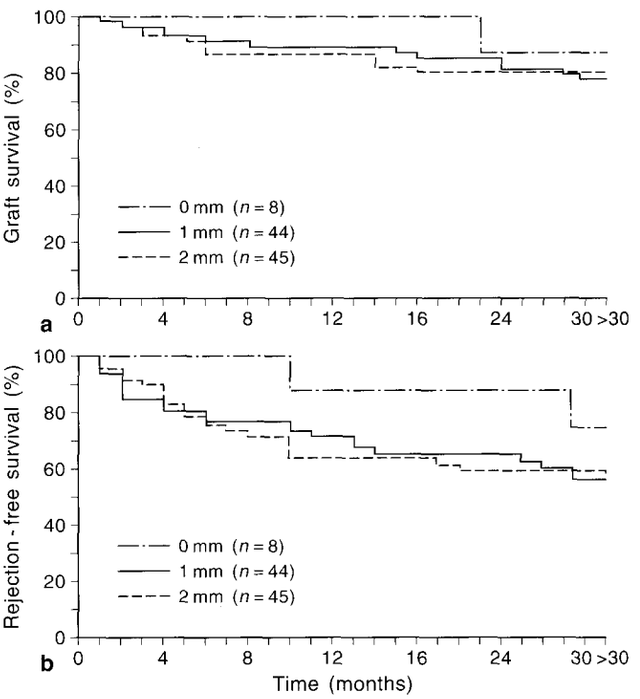

Fig. 1 a, b HLA-A locus: percentages of cumulative survival rates of patients with 0, 1, or 2 mismatches: **a** for graft failure; **b** for first graft reaction

Results

In the group of 115 recipients, 42 experienced at least one rejection episode and 20 experienced graft failure. The *P* values for Cox proportional hazard regression for all variables are shown in Table 1. Both the presence of a previous graft and a high degree of vascularization prior to transplantation were significant risk factors for an increased incidence of rejection episodes and for graft failure from rejection. The presence of ABO blood group incompatibility did not influence the incidence of the first rejection episode and had a slight beneficial effect on graft failure; neither results were significant (Table 1).

Matching for the HLA-A and the HLA-DR loci did not yield significant results (Table 1). Figure 1 shows the survival curves for the HLA-A locus. Although the number of patients with no mismatches was small, there is a slight beneficial effect of no mismatches over one or two mismatches. The curves for one and two mismatches closely follow each other. The results for the DR locus are depicted in Fig. 2. For both the incidence of the first rejection episode and graft failure from rejection, there is an advantage of no mismatches over one mismatch. However, patients with two mismatches fared better than those with one mismatch.

Surprisingly, the results for the HLA-B locus were completely different: the incidence of first rejection episode and graft failure from rejection showed a clear adverse matching effect. These results were statistically significant (Table 1). The results for graft failure are

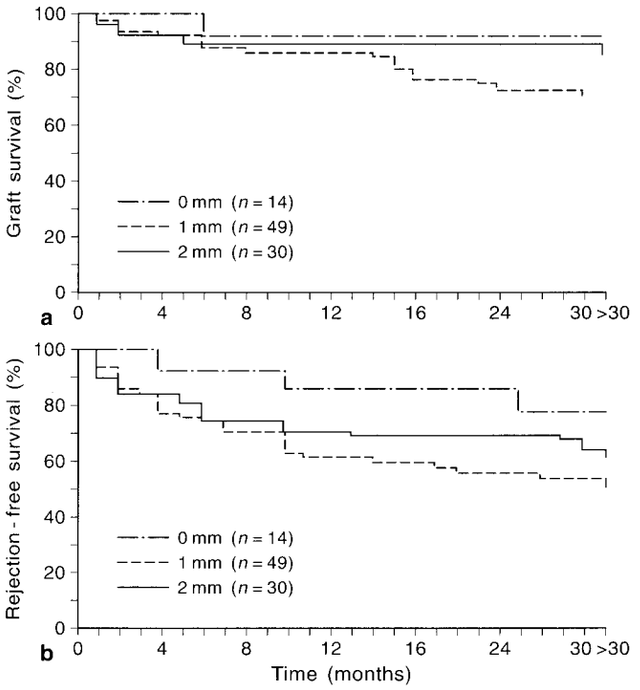

Fig. 2a, b HLA-DR locus: percentages of cumulative survival rates of patients with 0, 1, or 2 mismatches: **a** for graft failure; **b** for first graft reaction

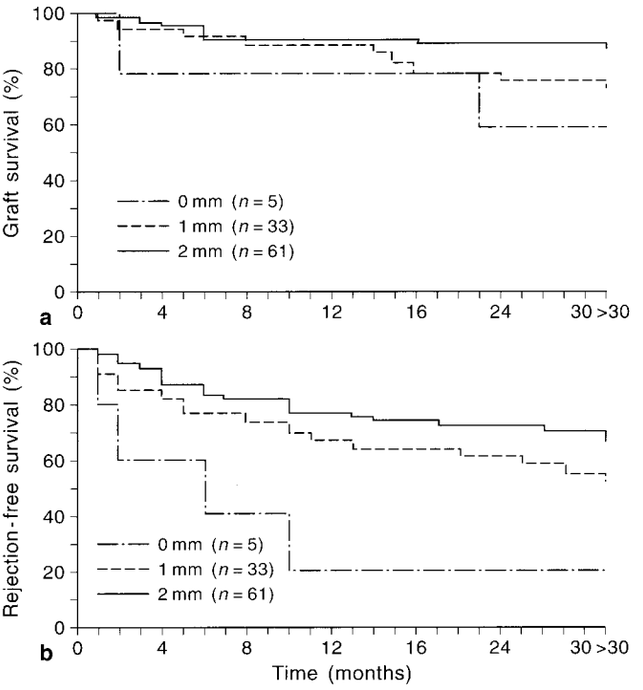

Fig. 3a, b HLA-B locus: percentages of cumulative survival rates of patients with 0, 1, or 2 mismatches: **a** for graft failure; **b** for first graft reaction

shown in Fig. 3 a, and the results for the incidence of first rejection episode are shown in Fig. 3 b. Both Fig. 3 a and Fig. 3 b show that the highest incidence of graft failure and first rejection episode occurred when there were no mismatches; the lowest incidence was observed when patients had two mismatches. The results for one mismatch were intermediate.

Discussion

Our study demonstrates that a previously rejected corneal graft or a high degree of vascularization significantly increases the incidence of both the first rejection episode and graft failure from rejection. These findings confirm the results from many previous reports. No consistent influence of ABO blood group compatibility on the incidence of rejection and graft failure could be observed and, in agreement with other investigators [2], we conclude that ABO incompatibility does not influence corneal graft survival.

The matching results for the HLA-A locus were similar to those commonly reported for transplantation. It has been frequently observed that one mismatch does not have much advantage over two mismatches [14], probably because one mismatch is sufficient to cause an immune reaction and rejection. The matching results for the HLA-DR locus, however, were somewhat unusual in that patients with two mismatches did better than patients with one mismatch. This effect was, however, not as strong as the clear adverse matching effect observed in the United Kingdom [19].

An interesting finding was the presence of a significantly adverse matching effect for the HLA-B locus. To our knowledge, an adverse matching effect for the HLA-B locus has not been reported earlier for any transplanted organ. In liver transplantation, HLA-DR matching appears to have an adverse matching effect [18]; viral agents have been shown to interfere with the HLA-DR matching results. Cytomegalovirus infections showed a much higher incidence in patients matched for the DR locus than for unmatched patients. In addition, matched patients had an increased incidence of chronic rejection [11]. The probability of a recurrence of hepatitis B or C virus infection has been found to be much greater when patients are matched for the HLA-B locus [12]. These findings support the proposed dualistic role of HLA in liver transplantation suggested by Markus et al. [13]. HLA matching reduces the incidence of acute rejection but permits allograft injury mediated by major histocompatibility complex (MHC)-restricted lymphocytes. Bradley et al. [3] hypothesize that the adverse HLA-DR matching effect observed in corneal transplantation is caused by presentation of alloantigen

or viral antigen by the donor cell DR locus to cytolytic T cells of the host. It is possible that the adverse effect of HLA-B matching reported in this study was caused by interference of a viral agent. Viral agents are known to evoke a strong HLA class I restricted cytolytic response mediated by CD8 + T lymphocytes [7, 22]. The killing of infected target cells in the cornea will result, but only in those patients who have been matched for the HLA class I molecule that presents the viral antigen to the T-cell receptor of the CD8 + T cell. Subsequent focal inflammation may both initiate and aggravate graft rejection. One candidate for a sensitizing viral agent is HSV, which infects and remains dormant in a large percentage of the general population and is also a well-recognized cause of recurrent clinical and subclinical infections in the eye. The trauma of surgery or immunosuppression after corneal transplantation may result in reactivation of HSV.

Both HLA-A and HLA-B molecules can present viral antigen. It is interesting to note that, in our study, the adverse matching effect did not occur at the HLA-A locus. Which type of HLA molecule presents the viral antigen may depend primarily on the type of antigen-processing pathway in the donor target cell [9]. Thus, the adverse matching effect of the HLA-DR locus on corneal grafts, observed in the United Kingdom [3, 19], may also be explained by infection with HSV. Geographical differences in HSV variants may determine which HLA loci present the viral antigen. The different genetic backgrounds of the population may also play a role in determining which antigen-processing pathway is used by the donor target cell.

The results of the Collaborative Corneal Transplantation Studies were analyzed for the HLA-A and -B loci by dividing them into "high match" (no or one antigen mismatch) and "low match" (two to four antigen mismatches) for both the HLA-A and HLA-B loci.

Thus, a positive matching effect in the HLA-A locus and a negative matching effect in the B locus could have added up to "no effect". It would be interesting to see whether other centers find a difference in effect between HLA-A and HLA-B matching on graft rejection and graft survival.

We suggest that HSV or another widespread virus may play an important role in corneal transplantation. If this is correct, then acyclovir or another antiviral agent should be included in the immunosuppressive protocol, especially in high-risk cases. Without such measures it may not be possible to establish the benefits of matching for HLA alleles in corneal transplantation.

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