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The Role of HLA-DRB1 Matching in Corneal Grafting

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A B S T R A C T

The aim of the study was to evaluate the role of HLA-DRB1 (Human Leukocyte Antigens) matching in corneal transplantation. Fifty-two patients were observed. Low-risk group consisted of 28 patients and high-risk group consisted of 24 patients. All the patients and donors were tissue typed with Polymerase Chain Reaction (PCR) on the HLA-DRB1 gen. The primary corneal disease preceding keratoplasty was keratopathia (15), leucoma (10), keratoconus (7), Re-KPP (6), impending perforation (4), combustio corneae (3), degenerative disorders (2), keratoglobus (1), keratouveitis (1), corneal maculae (1), and corneal melting syndrome (1). The graft rejection frequency was higher in the group of high-risk patients (29%) than in the group of low risk patients (7.1%). The rejection rate of compatible grafts was 37% for high risk and 2% for low risk group, while the rejection rate of incompatible was 44% in high risk and 5% in low risk group. We can conclude that HLA-DRB1 matching does not improve corneal graft survival.

Introduction

A transparence of cornea is a predisposing factor for clear and right picture on the retina. Unfortunately, there are many diseases that cause disturbances to the clear cornea and lead to the loss of vision. Corneal grafting is a method of choice in treating patients with such disturbances[1,2]. During years, corneal transplantation developed from a rough and risky experiment into a delicate and highly successful procedure. Today it represents one of the most successful transplantation procedures, with a 2-year survival rate in more than 90% of the initial grafts into avascular cornea[3]. Although the survival rate is over 90% in uncomplicated cases (keratoconus, degenerative disorders, keratoglobus, nonvascularized leukomas) the risk of graft rejection is between 65–70% in the high risk patients (previous graft rejection, intense vascu-

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larization of the recipient bed or previous inflammation)[3].

The extraordinary success of corneal transplantation is attributed to the »immunological privilege« of the cornea and the eye. From the immunological point of view, problems arise if the cornea is vascularized or if the recipient has a history of graft rejections[4–6].

Immunological privilege is a dynamic, physiological process that enables the eye to accept foreign tissue grafts for an extended, or even indefinite time. The maintenance of the immune privilege includes integrity of the blood-ocular barrier, absence of HLA class II (Human Leukocyte Antigens) molecule in the central cornea, phenomenon called ACAID (Anterior Chamber Associated Immune Deviation)[7]. The presence of blood-ocular barrier prevents specific and non-specific mediators of inflammation to gain access into the eye. Moreover, healthy cornea has no lymphatic drainage. Numerous studies have proved the presence of HLA class I antigen cells in all three corneal layers; however, HLA class II antigens are not normally expressed on corneal cells except on HLA class II bearing cells (Langerhans cells) which are absent from the central cornea and are situated at the corneal limbus[12].

Many recent researches have dealt with the problem of acceptance of corneal allografts. Because of the differences in opinions, some studies are being designed in order to evaluate the influence of HLA class II matching of recipient and cadaver on the survival of corneal grafts[3,4,8–11].

Patients and Methods

The research included 52 patients who had undergone corneal transplantation during the period 1997–1998 at the Department of Ophthalmology, General Hospital »Sveti Duh«. Thirty were men and 22 women. They were between 15–82 years of age. Risk factors are listed in Table 1. HLA typing was performed at the Tissue Typing Center, Urological Clinic, University of Zagreb, School of Medicine. It was performed on lymphocytes of the peripheral blood using PCR-SSOP (Polymerase Chain Reaction – Sequence Specific Oligo Probes). Probes used in PCR amplification were official, claimed at the XI International Workshop[12].

After surgery, all patients were given topical steroids (Dexamethason-Neomycin) four times a day, and steroid ointment two times a day for the first 2 postoperative months. Treatment was tapered according to local status.

TABLE 1
RISK FACTORS IN CORNEAL TRANSPLATATION

LOCAL RISK FACTORS
Vascularization of the recipient cornea
Preceding keratoplasty in the same or fellow eye
Preceding of the eye
Urgent surgery inflammation
Donor tissue of more than 8 mm in diameter
GENERAL RISK FACTORS
Blood transfusions
Preceding transplantations of other organs

High-risk patients (previous graft failure, inflammation, vascularization of the graft bed) and those having signs of immunological reaction (decreased graft clarity, endothelial precipitates, increased corneal thickness, epithelial rejection line, vascularization, inflammation) were treated with more invasive immunosuppressive regiment. If necessary, topical steroids were used every waking hour. In addition, patients with signs of severe reaction were given Cyclosporin A or Metilprednisolon oral or i.v. The initial dose

of Cyclosporin A was 10–15 mg/kg administered for the first 10–14 days, after which it was gradually reduced according to local status and blood levels until the maintenance dose of about 2–6 mg/kg given in two divided doses was reached. The initial dose of Metilprednisolon was 80 mg daily, and it was gradually reduced to the maintenance dose of 4-mg daily[13].

Statistical analysis was performed by χ^2 test and Fisher-direct test.

Results

The decision for corneal transplantation was made according to medical indications, after failure of other conservative and surgical treatments. Patients were divided according to corneal pathology detailed in Table 2.

TABLE 2
CORNEAL PATHOLOGY PRECEDING CORNEAL GRAFTING

Corneal pathology	No of eyes	%
Keratopathy	15	29%
Corneal scars	10	20%
Keratoconus	7	14%
Re-KPP	6	12%
Impending perforation	4	7%
Chemical burns	3	6%
Degenerations	2	4%
Keratoglobus	1	2%
Keratouveitis	1	2%
Corneal maculae	1	2%
Corneal melting syndrome	1	2%

The most common diagnosis for corneal grafting in our group of patients was keratopathy (15), than leucoma (10), and keratoconus (7). Re-keratoplasty was needed in 6 patients, impending perforation was present in 4 cases, 2 patients had degenerative corneal changes, 2 patients had keratoglobus cases, and 1 patient had keratouveitis. Macula of the cornea and cornea melting syndrome were each present in one patient. Table 3. shows the development of graft rejection.

TABLE 3
DEVELOPMENT OF GRAFT REJECTION

Appearance of the graft	No of grafts (%)
Clear graft	73%
Signs of graft rejection	10%
Irreversible graft rejection	17%

TABLE 4
THE RATE OF GRAFT REJECTION IN THE WHOLE (HLA-DRB1 COMPATIBLE+INCOMPATIBLE) GROUP COMPARED WITH THE REJECTION IN HLA-DRB1 INCOMPATIBLE GROUP

Presence of risk factors	HLA-DRB1 compatible + incompatible (%)	HLA-DRB1 incompatible (%)
High risk patients	29	31
Low risk patients	7	5

The success rates of corneal transplantations are given in Table 5. In 38 patients corneal graft remained clear, without signs of rejection. Although 5 recipients showed signs of immunological reaction, rejection was prevented by medication, and transplanted cornea remained clear. In 9 patients in spite of medications corneas were rejected.

In cases of rejection (14) all the recipients underwent screening for HLA antibodies in the blood. The antibodies found in two cases were not specific for antigens of donor corneas. As both patients belonged to the group of high-risk patients (previous blood transfusions and corneal transplantation) we concluded that the appearance of HLA antibodies represented a risk factor in graft rejection, but

TABLE 5
GRAFT REJECTION IN HLA-DRB1 COMPATIBLE AND INCOMPATIBLE PATIENTS OF THE HIGH AND LOW RISK GROUP

Presence of risk factors	HLA-DRB1 compatible (%)	HLA-DRB1 incompatible (%)
High risk patients	37%	44%
Low risk patients	2%	5%

was not a reliable sign to prove the immunnological reaction.

After risk factors were defined, patients were divided into a high risk group (24), and low risk group (28), in order to enable better analysis of the transplantation success. The frequency of rejection was analyzed according to risk factors (separately for the high risk and low-risk group of patients). The graft rejection frequency was higher in the group of high-risk patients (29%) than in the group of low risk patients (7.1%). Overall frequency of rejection (high and low risk patients together) was than compared to the frequency of the incompatible patients of the same group. In high-risk patients without HLA-DRB1 matched cornea the rejection rate was 31% compared to 5.2% in the low risk group of patients. The compared data showed that HLA-DRB1 allele incompatibility did not increase the risk of graft rejection (Table 4). As we can see in Table 5, there is no significant difference in the risk of graft rejection concerning HLA-DRB1 compatibilities between the two groups: 37% compared to 44% in the high-risk group, and 2% compared to 5% in the low-risk group.

Discussion

Disparity between a donor and recipient at the major histocompatibility complex (HLA in humans) is a predominant factor for allograft rejection. The recipient antibodies bound to the incompatible HLA of donor tissue increase the risk of accelerated allograft rejection. The major goal in transplantation surgery is to improve the donor-recipient histocompatibility, and to optimize the immunosupresive therapy[14].

The role of antigens encoded by the HLA in corneal grafting is controversial[15]. Some investigators claim that tissue typing correlates with the improved graft survival[8], whereas others have been unable to detect such effect[3].

This is in contrast to the beneficial effects of HLA-DR matching on the survival of other vascularized organs and may be explained by the lack of HLA antigen expression by native, uninflammed corneal tissue[16].

Collaborative Corneal Transplantation Studies (CCTS) Research Group designed the Crossmatch Study (CS) to assess the effect of crossmatching on corneal graft survival in high risk patients. The study demonstrates that neither HLA-A-B, nor -DR antigen matching substantially reduces the likelihood of corneal graft failure[8].

In CCTS study serological technique was used, disabling the detection of differences at the molecular level that led to graft rejection. For that reason we used the PCR technique in which the tissue typing is based on DNA material, and chose HLA-DRB1 gene, the most polymorphic gene in the HLA gene DRB subregion.

In a healthy cornea HLA class II bearing cells (Langerhans cells) are not present in the central cornea. These professional antigen-presenting cells are situated at the corneal limbus. No other corneal cells express HLA class II antigens, leading to decreased immunogenicity of the cornea[17]. Some studies have shown that heterotopically placed cor-

neas induce alloantigen specific cytotoxic T cells, but fail to induce a delayed type of hypersensitivity. However, the effect was lost when grafted corneas contained Langerhans cells[18].

In cases of vascularization (high-risk patients) this privilege is lost and donor cornea is infiltrated with host Langerhans cells that act as the Antigen Presenting Cells (APC) instead of the donor APC system and start an immunological reaction.

Unexpectedly, Corneal Transplant Follow-Up Study (CTFS) conducted in Great Britain showed that HLA-DR mismatched corneas had a significantly reduced risk of rejection. The authors suggested that corneal graft rejection might be mediated by a route involving recognition of allopeptides presented to CD4+ cells on compatible HLA-DR molecules[19]. This process differs from a strict definition of indirect pathway of recognition, which requires donor allopeptides to be processed by and presented on recipient APC. Instead, they have presumed that donor cells (e.g. endothelial cells) present donor allopeptides derived from HLA class I and minor antigens to host T cells. Endothelial cells carrying these target molecules are attacked by CD4+ host cells after docking onto compatible HLA-DR molecule. These findings have shown the beneficial effect of HLA-A-B matching but no such effect of HLA-DR matching in corneal transplantations. Studies on rats and mice have suggested a role of CD4+ cells in corneal allograft rejection[20]. Sonoda et al. (1992) have shown in an experimental model that usual immunogenetic rules for rejection do not apply, since minor disparate corneal grafts have shown higher rejection rate in comparison to the rejection rate of major (HLA) disparate grafts[21].

Our finding that HLA-DR matching does not improve corneal graft survival corresponds with the results of other studies[3,19]. Experimental models showed that extraordinary success of orthotopic corneal allograft survival is sustained by active immune regulatory system, which is multifactorial and results from alternation in both induction and expression of immune response in the eye[6].

The main goal of this research was to find the right way to create a state of specific tolerance to prevent graft rejection, as well as to find ways to restore elements of immune privilege in the high risk patients, in which this privilege is lost and consequently leads to much higher rejection rate. Restoration of the lost immune privilege or prevention of its abolition should optimize acceptance of corneal allografts. Experimental models show that the priority should be given to minimization of HLA class I mismatches, until further investigations finally give answer to the role of each HLA system in corneal grafting.

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ULOGA HLA-DRB1 PODUDARNOSTI KOD TRANSPLANTACIJE ROŽNICE

S A Ž E T A K

Cilj rada bio je istražiti ulogu HLA-DRB1 podudarnosti prilikom transplantacije rožnice. U istraživanje su uključena 52 pacijenta, od kojih je 28 spadalo u nisko-rizičnu a 24 u visoko-rizičnu skupinu primatelja. Davatelji i primatelji rožnica tipizirani su PCR metodom na HLA-DRB1 gen, te je utvrđen stupanj podudarnosti. Preoperativne dijagnoze bile su: keratopatija (15), leucoma (10), keratoconus (7), Re-KPP (6), prijeteća perforacija (4), combustia (3), degenerativne bolesti (2), keratoglobus (1), keratouveitis (1), macula rožnice (1) i rastapanje rožnice (1). Učestalost odbacivanja bila je veća kod primatelja rizične skupine (29%), nego kod nerizične skupine (7.1%). U skupini HLA-DRB1 podudarnih primatelja učestalost odbacivanja bila je 37% u skupini visoko-rizičnih i 2% kod nisko-rizičnih, dok je kod HLA-DRB1 nepodudarnih primatelja odbačeno 44% visoko-rizičnih i 5% nisko-rizičnih transplantata. Rezultati su pokazali da podudaranje primatelja i davatelj u HLA-DRB1 lokusu nije povećalo uspješnost preživljenja transplantata rožnice.