Profile of Cytokines in Aqueous Humor from Corneal Graft Recipients

Iva Dekaris, Nikica Gabrić, Renata Mažuran, Željka Karaman, Ivana Mravičić

Lions Croatian Eye Bank, Department of Ophthalmology, Holy Ghost General Hospital; and 1Institute of Immunology, Zagreb, Croatia

**Aim.** Due to the immune privilege of the anterior eye chamber, the success rate of corneal transplantation can reach 90%. The aim of this study was to determine cytokine profile in aqueous humor of patients undergoing corneal transplantation, and to establish whether cytokine profile at the time of surgery influenced corneal graft outcome.

**Methods.** Proinflammatory (TNF-β and IFN-γ) and immunosuppressive (TGF-β) cytokine levels were measured in aqueous humor and serum of 44 patients. Non-inflammatory corneal diseases included keratoconus (n=8), bullous keratopathy (n=7), and stromal dystrophy (n=3). Inflammatory diseases included corneal scars (n=10), rejection/ uveitis (n=1), infectious keratitis (n=1), and perforated ulcer (n=4). Control aqueous humor and sera were obtained from cadavers without corneal pathology.

**Results.** The concentration of TGF-β, in the aqueous humor in non-inflammatory corneal diseases was similar to that of controls (2,605 ± 204 pg/mL vs 2,200 ± 100 pg/mL). In inflammatory corneal diseases, the concentration of TGF-β, in aqueous humor was significantly lower (1,400 ± 375 pg/mL, p<0.001). TNF-β was detected in the aqueous humor of 16 out of 26 patients with inflammatory corneal diseases and in all patients with stromal dystrophies, but was undetectable in cases of keratoconus and bullous keratopathy. Low levels of IFN-γ were present in all aqueous humor samples. Patients’ sera contained significantly less cytokine (up to 252 pg/mL) than their aqueous humor (p<0.001). We have set an arbitrary cut-off point for TGF-β, level in aqueous humor at 1,500 pg/mL and divided all investigated samples (from 44 patients and 10 controls) into two groups, one with high and the other with low TGF-β, concentration. The coefficient of contingency showed that patients with high TGF-β, concentration in their aqueous humor had significantly greater chance for graft acceptance than those with low TGF-β, concentration (p<0.001).

**Conclusion.** High TGF-β, concentrations in the eyes without intraocular inflammation suggest its immunosuppressive role in human eyes. High concentration of TGF-β, (>1,500 pg/mL) was associated with graft acceptance. Also, absence of proinflammatory TNF-β increased the graft acceptance, but independently from TGF-β,.

**Key words:** aqueous humor; corneal disease; corneal transplantation; cytokines; eye diseases; graft survival; immune tolerance; immunosuppression; inflammation; interferon type II; transforming growth factor; tumor necrosis factor

Cytokines are short-lived molecules that act primarily in the local milieu in picomolar to nanomolar concentrations to regulate host cell function. Since they are effective at very low concentrations, small changes in their concentration may result in profound changes in the immunoinflammatory response. The intraocular microenvironment is an immune-privileged site where immunogenic inflammation has been associated with immunosuppressive factors found in aqueous humor produced by ocular tissues. For example, transforming growth factor-beta (TGF-β) can suppress immune cell activity, as shown in the anterior chamber of the eye (1). The abrogation of the intraocular immune privilege is associated with the presence of proinflammatory cytokines, e.g., in uveitis or allograft rejection (2-9). It seems that the character of immune response at specific site may be determined by the set of released cytokines (10).

Immune privilege of the anterior eye chamber was recorded over 100 years ago, when researchers found that xenogeneic tumor grafts survived significantly longer in the anterior chamber than at other sites (11-13). The first hypothesis trying to explain the phenomenon was that the antigens placed in the anterior chamber were sequestered, and therefore the different blockade of the immune system was present (11). However, it has become clear that antigens placed in the eye had the access to the systemic circulation (13,14), and that the graft survived due to the immunoregulatory processes within the anterior chamber, which resulted in the suppression of the delayed type hypersensitivity to the antigen introduced through the anterior chamber (12,13). The phenomenon where the inoculation of the antigen via the anterior chamber depresses the cell-mediated immune response to that specific antigen is called anterior chamber-associated immune deviation (15). Cytokines are mediators of the immunosuppressive properties of the anterior eye chamber. Streilein and co-workers...
(16) have shown that TGF-β1 plays the critical role in the induction of anterior chamber-associated immune deviation and that it is present in all three fluids that confer anterior chamber-associated immune deviation-inducing properties, in vitro: aqueous humor, cerebrospinal fluid, and amniotic fluid (17).

Despite the immune privilege of the anterior eye chamber, allograft rejection remains the main cause of corneal graft failure. This is particularly true in the eyes with concurrent inflammatory disease, where the rejection rate may exceed 50% (18). This occurs because immune privilege can be abrogated in neovascularized corneas, like in ocular inflammation, and in the presence of Langerhans cells in the central cornea (19-22). Recent experimental studies have implicated proinflammatory cytokines as mediators of graft rejection of non-ocular tissue. For example, in allografts such as heart, liver, and kidney, cytokines mediate alloimmune response (23,24). Although the molecular mechanisms of ocular allograft rejection remain poorly understood, there is rising evidence in animal models that proinflammatory cytokines can modulate the immune response to corneal graft (6,7,25-27). To explore whether the same might be true for humans, we investigated the cytokine profile (interferon-γ, TNF-β, and TGF-β2) in the aqueous humor of corneal graft recipients suffering from different corneal diseases.

Patients and Methods

Patients

Cytokine levels were measured in the aqueous humor obtained from 44 patients during corneal transplantation. Patients with any inflammatory disease apart from corneal pathology were excluded from the study. Median age of the patients was 61 (range, 19-83); 19 were women and 25 men. Patients were divided into two groups according to the presence of inflammation in their anterior eye segment. The first group included patients with non-inflammatory corneal diseases: eutactic corneal dystrophies (n=8), bullous keratopathy (n=7), and stromal corneal dystrophy (n=3). The second group included inflammatory corneal diseases such as postkeratoclastic vascularized corneal scars (n=10), corneal graft rejection (n=5), pending perforation caused by corneal ulcer (n=4), corneal scar caused by chemical burn (n=4), graft rejection and active uveitis (n=1), infectious keratitis (n=1), and perforated corneal ulcer (n=1).

Aqueous Humor Collection

Aqueous humor was collected before corneal transplantation through the paracentesis performed at 11 o’clock position with a 1-mL syringe and 25-gauge needle. Anterior chamber depth was restored with a viscoelastic solution (Viscoat, Alcon, Forth Worth, TX, USA) and the surgery continued in a standard manner. The same surgeon used the same operative technique performed all the surgeries. The process of aqueous humor collection was in accordance with the standards of the hospital ethical committee. Collected aqueous humor samples were immediately stored at -21°C.

Control Aqueous Humors

Control aqueous humors (n=10) were obtained from cadaver eyes in Lions Croatian Eye Bank (not later then 2 h after death) unsuitable for corneal transplantation because of the small endothelial cell count or patient’s medical history. Small endothelial cell count has never been connected with intraocular cytokine level (18) and special effort was given to select samples only from the patients with previous illnesses unrelated to the eye. Cadavers with any known inflammatory disease were excluded from the study. After retrieval of the donor globe, standard disinfecting procedure was performed: vigorous irrigation with tap water and immersion of globe into 2% povodone-iodide, followed by irrigation with sterile saline containing gentamycin. Globes were examined under a slit-lamp. Aqueous humor samples were retrieved under sterile conditions from the globes with low corneal endothelium cell counts, in the same manner as during the surgery. Samples were immediately stored at -21°C.

Patient Sera

To determine systemic cytokine production, blood samples were collected from all patients at the time of surgery. Serum samples were stored at -21°C until analysis. Control sera were obtained from the 10 cadavers whose aqueous humors were used as controls.

Cytokine Quantitation

Aqueous humors were analyzed by enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer instructions. TNF-β, IFN-γ, and TGF-β2 kits were purchased from Quantikine, R&D Systems, Minneapolis, MN, USA.

Association of Cytokine Level and Clinical Signs of Graft Reaction

Patients were followed-up for at least six months after surgery and each clinical sign of corneal graft reaction was recorded. All patients received only topical steroid-antibiotic treatment. Clinical signs of corneal graft reaction were the following: (a) epithelial rejection—appearance of the epithelial rejection line representing the zone of destruction of donor epithelial cells; (b) subepithelial infiltrates seen in the graft; (c) sudden onset of stromal edema and haze in previously clear graft; and (d) presence of endothelial rejection line or diffuse keratic precipitates on endothelium.

Diagnosis of allograft reaction was made only in technically successful grafts that had remained clear for at least 10-14 days after corneal transplantation. Cytokine levels found in aqueous humor on the day of surgery were associated with the presence of mentioned signs in a postoperative period. Despite one or more episodes of graft reaction, some grafts would finally clear after standard administration of topical and systemic steroid treatment, and these were considered accepted. Corneal grafts, which had clinical signs of graft reaction and have never cleared, were considered rejected (or failed).

Statistical Analysis

Comparison between multiple groups was performed by Kruskal-Wallis test (aqueous humor concentration of each cytokine among different groups), and between two groups by Student’s t-test (aqueous humor versus sera inside each group). Probability of <0.001 was considered statistically significant.

Results

TGF-β2 Profile in Aqueous Humor of Patients with Different Corneal Diseases

The presence of immunosuppressive cytokine TGF-β2 was assessed in aqueous humors of patients suffering from different corneal diseases. The mean production of TGF-β2 in aqueous humor of the eyes with inflammatory corneal diseases (1,400 ± 375 pg/mL) was significantly lower than that in non-inflammatory corneal diseases (2,605 ± 204 pg/mL) and controls (2,200 ± 100 pg/mL) (p < 0.001, Fig. 1). There was no statistically significant difference in aqueous TGF-β2 concentrations between non-inflammatory corneal diseases and controls. We then tested whether TGF-β2 could also be found in the patients’ sera. TGF-β2 amount in all sera was significantly lower then in the aqueous humor for all investigated groups (p < 0.001), independently of the presence of an ocular inflammatory process.
Proinflammatory Cytokine Profile in Patient’s Aqueous Humor

Although TGF-β concentrations differed among the eyes with and without ocular inflammation, this cytokine could be found in all investigated samples (Fig. 1). Proinflammatory cytokines, however, were found only in specific corneal diseases and their concentration (10-96 pg/mL) contrasted sharply with high concentration of immunosuppressive cytokine TGF-β (at least 1,400 pg/mL). For example, in inflammatory corneal diseases, TNF-β was detected in aqueous humor of all patients with previous graft rejection, chemical burns, infectious keratitis, and perforated ulcer, and in half of the patients with post-keratitic vascularized corneal scar (altogether 16 out of 26 inflamed eyes). In other inflammatory corneal diseases, such as pending perforation caused by ulcer, rejection in the uveitic eye, and post-keratitic vascularized scars, TNF-β could not be detected (altogether 10 out of 26 inflamed eyes). Unexpectedly, in 3 patients with stromal corneal dystrophy, aqueous TNF-β amount was similar as in eyes with inflammation (p=0.216). In control aqueous humors, the concentration of TNF-β was significantly lower then in the eyes with stromal dystrophy or inflammation (p<0.001). TNF-β was undetectable in the patients sera (Fig. 2). Aqueous humor of patients with other investigated non-inflammatory corneal diseases (keratoconus and bullous keratopathy) had no detectable level of TNF-β.

Small amounts of IFN-γ could be detected in all inflammatory corneal diseases and, like the other proinflammatory cytokine TNF-β, in the aqueous humor of eyes with corneal dystrophy (Fig. 3). There was no significant difference in IFN-γ concentrations between these two groups (p=0.529). However, IFN-γ was also present in all control samples. In all groups, the serum concentration of this proinflammatory cytokine was significantly lower then in aqueous humor (p<0.001).

Association of Cytokine Concentration in the Aqueous Humor with Clinical Outcome of the Corneal Graft

The patients were followed-up for 6 months after surgery and all received standardized postoperative topical steroid-antibiotic treatment. We aimed to determine whether the profile of examined cytokines at the time of surgery had any impact on the acceptance rate of corneal grafts. The presence of any clinical sign of graft reaction (despite the final graft outcome) was established by the slit-lamp examination performed and recorded by an experienced corneal surgeon. Grafts were considered rejected in the case of irreversible graft reaction (unresponsive to daily steroid treatment with peribulbar dexamethason injections). Although a whole spectrum of cytokines can influence the outcome of graft following surgery, we investigated whether the cytokines analyzed in our study could have any predictive value for a grafted patient.

Figure 1. TGF-β concentrations (mean±SD) in the aqueous humor and serum of corneal graft recipients suffering from non-inflammatory (n=18) and inflammatory corneal diseases (n=26). Open bars – TGF-β concentration in the aqueous humor; closed bars – TGF-β concentration in serum. Asterisk indicates a statistically significant difference in TGF-β concentration in aqueous humor between the inflammatory group and the non-inflammatory and control group (p<0.001). Double asterisks indicate a statistically significant difference in TGF-β concentration between the aqueous humor and serum within each tested group (p<0.001).

Figure 2. TNF-β concentrations (mean±SD) in the aqueous humor and serum of corneal graft recipients suffering from stromal corneal dystrophy (n=3) and inflammatory corneal diseases (n=16). Open bars – TNF-β concentration in the aqueous humor; closed bars – TNF-β concentration in serum. Asterisk indicates a statistically significant difference in TNF-β concentration in aqueous humor between the stromal dystrophy and inflammatory group and the control group (p<0.001). Double asterisks indicate a statistically significant difference in TNF-β concentration between the aqueous humor and serum within each tested group (p<0.001).
Proinflammatory cytokines TNF-β and IFN-γ have been implicated to play a role in allograft rejection of other human organs and animal corneal allografts (6,23,24). IFN-γ was detected in both control and diseased eyes and therefore ruled out as a possible predictor of corneal graft rejection. Thus, our prospective study focused on patients whose aqueous humor contained measurable amounts of proinflammatory cytokine TNF-β (16 patients with inflammatory corneal disease and 3 patients with stromal corneal dystrophy). In this selected group of TNF-β positive patients with inflammatory corneal disease (Table 1), the rejection rate was 62% (10 out of 16), which is higher then the overall rejection rate in the inflammatory group (46%, or 12 out of 26).

Higher levels of TNF-β were not inevitably connected with graft failure (Table 1). However, concomitant presence of low-levels of TGF-β₂ (<1,500 pg/mL) in these patients seemed to be closely associated with graft failure. For example, in two patients with post-keratitic scars having same amount of TNF-β (68.4 pg/mL) and different levels of TGF-β₂ (1,252 vs 2,225 pg/mL), corneal graft had failed only in the recipient with low amount of TGF-β₂, suggesting immunoprotective role of this cytokine in human eyes (Table 1). Therefore, for the further analysis, we have set the arbitrary cut-off point for TGF-β₂ level in aqueous humor at 1,500 pg/mL and divided all investigated samples (from 44 patients and 10 controls) into two groups, one with high and the other with low TGF-β₂ concentration (Table 2). The coefficient of contingency (Table 2) showed that patients with high (≥1,500 pg/mL) TGF-β₂ concentration in their aqueous humor had significantly greater chance for graft acceptance than those with low (<1,500 pg/mL) TGF-β₂ concentration (p<0.001).

Out of 26 patients with inflammatory corneal diseases, 16 had detectable concentration of TNF-β in their aqueous humor (Table 1), whereas the remaining 10 did not. Only two of these TNF-β negative patients rejected their graft, and both of them had less then 1,500 pg/mL of TGF-β₂ in their aqueous humor. The other eight TNF-β negative patients accepted corneal graft despite low TGF-β₂ concentration (mean±SD, 1,241±308 pg/mL).

**Table 1.** Clinical outcome of corneal grafts placed in eyes (n = 19) with proinflammatory cytokine TNF-β in their aqueous humor (AqH)

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of patients</th>
<th>Cytokines (pg/mL) in AqH</th>
<th>Graft outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stromal dystrophy</td>
<td>3</td>
<td>74.4</td>
<td>2608 C</td>
</tr>
<tr>
<td>(Mb. Groenouw type I)</td>
<td></td>
<td>62.0</td>
<td>2720 C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84.2</td>
<td>2540 C</td>
</tr>
<tr>
<td>Post-keratitic vascular scar</td>
<td>5</td>
<td>68.4</td>
<td>1252 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>71.2</td>
<td>1440 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.2</td>
<td>1280 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.4</td>
<td>1980 C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>68.4</td>
<td>2225 C</td>
</tr>
<tr>
<td>Previous graft rejection</td>
<td>5</td>
<td>46.7</td>
<td>2175 C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>89.3</td>
<td>1480 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.4</td>
<td>2180 C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72.4</td>
<td>1260 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60.4</td>
<td>1331 F</td>
</tr>
<tr>
<td>Chemical burns</td>
<td>4</td>
<td>90.4</td>
<td>1650 C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>62.6</td>
<td>1390 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>112.2</td>
<td>1368 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>53.4</td>
<td>1202 F</td>
</tr>
<tr>
<td>Infectious keratitis</td>
<td>1</td>
<td>90.8</td>
<td>1400 F</td>
</tr>
<tr>
<td>Perforated ulcer</td>
<td>1</td>
<td>55.4</td>
<td>1500 C</td>
</tr>
<tr>
<td>Healthy eyes (controls)</td>
<td>10</td>
<td>0</td>
<td>2,200 ± 100</td>
</tr>
</tbody>
</table>

C – clear graft; F – failed graft.

**Table 2.** TGF-β₂ concentration in patient’s aqueous humor at the time of corneal transplantation

<table>
<thead>
<tr>
<th>TGF-β₂ (pg/mL)</th>
<th>no-inflammatory</th>
<th>inflammatory</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1,500</td>
<td>18</td>
<td>8</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>&lt;1,500</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>26</td>
<td>10</td>
<td>54</td>
</tr>
</tbody>
</table>

χ² = 29.07, p<0.001.

**Discussion**

It is still unclear whether TGF-β mediates ocular immune privilege in human eyes, as it has been found in animals (1,12,13). Our data show that this cytokine can be detected in human aqueous humor of both diseased and control eyes. Significantly lower serum concentrations of TGF-β₂ indicated that it was produced by ocular tissues. However, the concentration...
of TGF-β, significantly differed between patients with inflammatory diseases on the one hand, and those with non-inflammatory corneal diseases and controls, on the other. In non-inflammatory corneal diseases, TGF-β concentration was similar to the control samples, suggesting that intraocular microenvironment in these eyes remained immunosuppressive. Our data speak in favor of the general hypothesis that TGF-β mediates immune privilege and consequently has a protective role regarding allotransplantation (12, 13). This might, at least partly, explain clinically observed high allograft success rate of over 90% in patients with non-inflammatory corneal diseases (18). Other authors have shown that high success rate of corneal transplantation can also be attributed to the lack of antigen-presenting cells in the central corneal epithelium, lack of blood and lymphatic vessels in the cornea, and presence of several factors suppressing the immune and inflammatory reaction within the anterior eye segment (28-33). The role of TGF-β in the aqueous humor and vitreal fluid was investigated in both aqueous humor and vitreal fluid of inflamed and non-inflammatory corneal diseases (18). Other authors have shown that high success rate of corneal transplantation can also be attributed to the lack of antigen-presenting cells in the central corneal epithelium, lack of blood and lymphatic vessels in the cornea, and presence of several factors suppressing the immune and inflammatory reaction within the anterior eye segment (28-33). The role of TGF-β in the aqueous humor and vitreal fluid was investigated in both aqueous humor and vitreal fluid of inflamed eyes than in controls.

There are two isoforms of TGF-β. TGF-β1 is responsible for most of the biologic activity in rabbit and human aqueous humor (80-90%) (33). Streilein and co-workers (33) showed that significant amounts of TGF-β1 (100-1,000 pg/mL) can be found in healthy eyes. Jampel et al (35) have detected TGF-β in all aqueous humor samples from the eyes of patients undergoing cataract surgery and its amount ranged from 2,300 to 8,100 pg/mL, with 61% of TGF-β in the active form. The concentration of TGF-β in the aqueous humor is sufficient to inhibit T cell activation and proliferation (1).

The characteristics of the ocular microenvironment depend on more than one cytokine. Since increased Th1 cytokine levels in animal aqueous humor correlate well to corneal graft rejection (6), we investigated whether these cytokines could be found in aqueous humor of patients undergoing corneal transplantation. IFN-γ and TNF-β are both secreted by Th1 cells, and have been implicated in allograft rejection of human organs (23, 36). These factors were chosen for three reasons: (a) in orthotopic mouse after corneal transplantation, Th1 cytokine expression was constantly predominant in the aqueous humor and rejected corneal grafts (6); (b) IFN-γ is a major pro-inflammatory cytokine released by Th1, which also has the ability to abrogate intraocular immune privilege (8); and (c) TNF-β acts proinflammatory by playing the critical role in lymphoid development and activation of polymorphonuclear leukocytes, inducing MHC expression and prostaglandin synthesis (37, 38).

According to our results, human aqueous humor concentration of IFN-γ is either very low (in patients with inflammatory corneal diseases or stromal corneal dystrophy and in controls) or undetectable (in keratoconus and bullous keratopathy). This is at least true for the later stages of inflammatory corneal disease, when corneal transplantation is needed. Limitation of our study was that we only measured IFN-γ concentration in aqueous humor immediately before corneal transplantation, and therefore had no data on local production of this cytokine at other stages of corneal disease or after corneal grafting.

As opposed to IFN-γ, we have detected TNF-β in most aqueous humors from the eyes of patients with inflammatory corneal diseases, suggesting that this cytokine might act pro-inflammatory. Other authors have shown that the so-called proinflammatory cytokines lead to the amplification of inflammatory responses through the induction of chemokines, activation of antigen-presenting cells, and increased expression of adhesion and co-stimulatory molecules (39-41). Although both cytokines are produced locally by T-cells, the discrepancy between TNF-β and IFN-γ in our findings might be that further evidence of tissue destruction and secretion of each cytokine at different stages of corneal disease. TNF-β was not found in the aqueous humor of all patients with ocular inflammation. For example, only half of the eyes with vascularized post-keratitic corneal scars contained measurable concentration of TNF-β in their aqueous humor, showing that patients who would be clinically placed in the same group for graft prognosis might have completely different inflammatory processes in their anterior chamber (at least when TNF-β is concerned). In these, highly vascularized corneas, graft rejection rate in TNF-β positive (3 out of 5) and TNF-β negative (2 out of 5) eyes was similar. It seems likely that in TNF-β negative aqueous humor some other proinflammatory cytokines mediate graft rejection development.

We detected high amounts of TNF-β in the aqueous humor of patients with corneal dystrophy, which was unexpected because corneal dystrophy is usually not connected with ocular inflammation. This is the first time ever that the presence of TNF-β in the aqueous humor of human eyes with corneal dystrophies has been shown. Since all grafts remained clear, one might assume that proinflammatory cytokine TNF-β in these eyes had no influence on graft rejection and that high concentration of TGF-β2, which was detected in such eyes, gave sufficient protection against graft rejection. However, our research included a small group of patients.

Previous studies showed that different local mechanisms governing the balance of cytokines in the aqueous humor might influence further development of immune reaction toward grafted tissue (6, 7, 25, 26). Maybe the analysis of proinflammatory and immunosuppressive cytokine levels at the time of surgery can help in a prognosis of graft success. We have started form the hypothesis that the balance of cytokines present in the anterior chamber can determine the outcome of the corneal graft depending on whether proinflammatory cytokines promote the conventional pathway of immune reaction or immunosuppressive cytokines maintain the specific characteristic of the eye as a privileged site. Our results show that increased concentration of TNF-β did not invari-
ably lead to graft rejection. However, it may be a stimulating factor for rejection because in TNF-β positive patients with inflammatory corneal diseases the graft rejection rate was 62% (10 out of 16), vs only 20% (2 out of 10) in TNF-β negative eyes. Proinflammatory IFN-γ could not influence this difference because it was detected in both diseased and control eyes. On the contrary, the concentration of immunosuppressive TGF-β2 was not only significantly different between inflammatory, non-inflammatory, and control aqueous humor (Fig. 1), but also considerably varied among patients with the same clinical diagnosis. Therefore, the concentration of TGF-β2 and TNF-β in aqueous humor were compared among patients that had both cytokines in their aqueous humor when they underwent corneal transplantation. The outcome of the grafts was followed up for the next 6 months. We showed that high TGF-β2 concentration in aqueous humor at the time of corneal transplantation significantly increased the chance of graft survival, whereas TGF-β2 below 1,500 pg/mL invariably led to graft failure (Table 2).

In TNF-β negative patients, corneal grafts were mainly accepted, both in non-inflammatory (with high TGF-β2 concentration), and in inflammatory diseases (mean TGF-β2 of only 1,200 pg/mL), implicating a generally better prognosis for a graft in the absence of TNF. Even when proinflammatory cytokine, such as TNF-β, is present in aqueous humor, our results suggest that there is a critical level of TGF-β2 that will preserve immune privilege and decrease chance of corneal graft rejection. Therefore, it seems that TGF-β2 has a role in the maintenance of ocular immune privilege even in human eyes. However, further research, which would include the whole pattern of cytokines and more patients, is needed to completely understand the roles of cytokines in corneal grafting.

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Correspondence to:
Iva Dekaris
Lions Croatian Eye Bank
Department of Ophthalmology
Holy Ghost General Hospital
Sveti Duh 64
10 000 Zagreb, Croatia
iva.dekaris@eeba.net