Influence of Interleukin-1α and Tumor Necrosis Factor-α Production on Corneal Graft Survival

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Aim To determine pro-inflammatory cytokine secretion from human corneas with different pathology and to establish whether cytokine profile influences corneal graft outcome.

Method Secretion of both proinflammatory cytokine interleukin (IL)-1α and tumor necrosis factor (TNF)-α was measured after cultivation of 47 corneas collected from corneal graft recipients suffering from different corneal diseases. Non-inflammatory corneal diseases were keratoconus (n = 8), keratoglobus (n = 2), bullous keratopathy (n = 11), and Groenouw stromal dystrophy type II (n = 2), whereas inflammatory included vascularized corneal scar (n = 14), rejected graft (n = 6), and corneal ulcer (n = 4). Corneas were cultivated at 37°C for 24 hours and frozen until cytokine detection was measured by immunoassay. Donor corneas unsuitable for transplantation were used as controls (n = 7). Corneal graft recipients were followed at least 18 months and rejection rate was calculated for each group.

Results The median concentration of IL-1α secreted from corneas of recipients with non-inflammatory diseases was 2.47 pg/mm^3 (range, 0.13-9.95). In inflammatory corneal diseases, IL-1α concentration was significantly higher (median, 5.92 pg/mm^3; range, 0.48-12.68; $P = 0.005$). IL-1α production in controls (median, 0.63 pg/mm^3; range, 0.36-1.29 pg/mm^3) was significantly lower than in inflammatory corneal diseases ($P<0.001$) and non-inflammatory diseases ($P = 0.008$). Low level of TNF-α was detected only in 5 cases of vascularized corneal scars, 3 cases of bullous keratopathy, and 3 cases of graft rejection. Rejection rate was significantly higher in inflammatory than in non-inflammatory group (46% vs <10%, respectively, $P = 0.008$). IL-1α and TNF-α were absent from all patient's sera, confirming its local intra-ocular production.

Conclusion Increased production of IL-1α in corneal recipients with inflammatory diseases suggests its role in corneal graft rejection in humans.

The intraocular microenvironment is an immune-privileged site where immunogenic inflammation has been associated with immunosuppressive factors found in aqueous humor. The abrogation of the intraocular immune privilege is associated with the presence of proinflammatory cytokines in uveitis (1,2) or allograft rejection (3,4). It seems that the character of immune response at this specific site may be determined by the set of released cytokines (5-7).

The first hypothesis trying to explain the immune privilege of the anterior eye chamber was that the antigens placed in the anterior chamber were sequestered, which was the reason for the afferent blockage of the immune system (8). However, it has been found that antigens placed in the eye reach systemic circulation and prolonged survival of allografts in the anterior chamber has been explained by suppression of the delayed type hypersensitivity to the antigen introduced through the anterior chamber (9,10). The phenomenon where the inoculation of antigen via the anterior chamber depresses the cell-mediated immune response to that specific antigen is called anterior chamber-associated immune deviation (11).

In animal models, transforming growth factor (TGF)-β_2 was shown to play a critical role in the induction of anterior chamber-associated immune deviation (12,13). It was found in all free fluids that confer properties inducing anterior chamber-associated immune deviation in vitro, ie, in aqueous humor, cerebrospinal liquid, and amniotic fluid (12,13). However, in animal models, the phenomenon of anterior chamber-associated immune deviation is lost after corneal transplantation or when corneal inflammation is present (14).

Although the mechanisms of maintenance and loss of anterior chamber-associated immune deviation in the mouse eye are well explained, as in case of allograft transplantation, there are limited data on immune mechanisms following allograft transplantation in the human eye. In our previous study, we found that high concentration of TGF-β_2 and the absence of proinflammatory tumor necrosis factor (TNF)-β was associated with high graft-acceptance rate in human eyes with non-inflammatory corneal diseases (15). This finding suggested that TGF-β_2 might promote corneal graft survival also in human eyes, and even establish a form of anterior chamber-associated immune deviation in eyes with non-inflammatory corneal diseases.

Despite the immune privilege of the anterior chamber in the healthy eye, allograft rejection remains the main cause of corneal graft failure because immune priviledge is lost in diseased corneas (16). This is particularly true in eyes with present inflammation, where the rejection rate may exceed 50% (17). This occurs because immune privilege can be abrogated in several ways, like in ocular inflammation, in the presence of Langerhans cells in central cornea, and after keratoplasty (14,18-20).

Experimental studies have implicated proinflammatory cytokines as mediators of graft rejection in non-ocular tissue. In allografts such as heart, liver, or kidney, IL-1α and TNF-α mediate alloimmune response (21,22). Although the molecular mechanisms of ocular alloimmunity remain poorly understood, animal models provide evidence that proinflammatory cytokines, such as interleukin (IL)-1α and TNF-α, can modulate immune response to the corneal graft (3,4,23-27). Moreover, local suppression of cytokine activity by their antagonists, such as interleukin-1 receptor antagonist (IL-1ra) and soluble TNF receptor, promotes corneal graft survival (28-32). Having in mind a significant benefit from such local treatment in the prevention of graft rejection in human eyes, we decided to determine whether IL-1α and TNF-α were produced by human corneal graft recipients similarly as they were in the animal model.

Subjects and methods

Subjects

Corneal tissue buttons were obtained from 47 patients with various corneal diseases who

were surgically treated at our Department from 2001 to 2003. Informed consent was obtained from all patients after the explanation of the nature of the study. The study included 20 men and 27 women, aged 20-70 years. Patients were divided into two groups according to the presence of inflammatory signs at the time of surgery. One group included patients with inflammatory corneal diseases, as follows: vascularized corneal scar (n = 14), rejected graft (n = 6), and corneal ulcer (n = 4). The other group included patients with non-inflammatory corneal diseases, as follows: bullous keratopathy (n = 11), keratoconus without hydrops (n = 8), keratoglobus (n = 2), and Groenouw stromal dystrophy type II (n = 2). Donor corneas unsuitable for transplantation were used as controls (n = 7). The inclusion criteria for "inflammatory" group were the presence of one or more clinical signs of inflammation, such as neovascularization of the cornea, infiltration of the corneal stroma, and corneal ulcer. Although different amount of inflammation can be observed at different time in each disease, our patients with the same diagnosis had a clinically similar degree of inflammation at the time of surgery.

Human tissue used in these experiments was obtained and managed in accordance with the provisions of the Declaration of Helsinki (33).

Tissue and sera collection

Preoperatively, thickness of each affected and control cornea was measured by ultrasound pachymetry (Alcon Surgical 8700, Alcon, Forth Worth, TX, USA). Corneal and serum samples were collected from graft recipients during corneal transplantation. Since we surgically treat our patients at approximately similar stage of disease, depending on the availability of the donor tissue, the duration of corneal disease in each patient was similar but not equal. Diameter of each excised corneal sample was recorded after trephination. Corneal buttons were immediately cultivated for 24 hours at 37°C in 0.5 mL of corneal storage media (CorneaPrep, EuroBio, Les Ulis, France). After cultivation, corneas and their cultured supernatants were frozen and kept at -21°C until cytokine detection.

Control corneas were obtained from healthy cadavers, with the informed consent of family members according to the hospital protocol. Only corneas that were not suitable for transplantation due to small number of endothelial cells were used.

Cytokine quantitation

Commercial kits for enzyme-linked immunosorbent assay (ELISA; Quantikine, R&D Systems, Minneapolis, MN, USA) were used according to the manufacturer's instructions to determine concentrations of TNF-α and IL-1α in supernatants of cultivated corneas and in sera. ELISA assays were repeated twice for each cornea. Cytokine concentration was calculated and adjusted for each cultivated cornea depending on its volume according to the following formula: $r^2 \times \pi \times h$ (r – corneal diameter, $\pi = 3.14$, h – corneal thickness), and expressed per mm^3 of tissue. Due to the fact that corneal thickness might be influenced not only by higher number of corneal cells but also by hydration of the cornea (due to dysfunctional endothelial cells), the results were also shown as cytokine concentration per mL of supernatant (without adjustment for corneal thickness). Results were presented for overall cytokine production per group (inflammatory and non-inflammatory) and cytokine concentration per each corneal disease.

Association of cytokine concentration and clinical signs of graft reaction

Corneal graft recipients that donated their diseased corneas for this experiment were followed up for at least 18 months after surgery and each clinical sign of graft reaction was recorded. 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 a previously clear graft; and (*d*) presence of the endothelial rejection line or diffuse keratic precipitates on endothelium. Diagnoses of allograft reaction were made only in technically successful grafts that had remained clear for at least 10-14 days after corneal transplantation, or 7 days in case of previous rejection. Cytokine concentrations found in corneas on the day of surgery were associated with the presence of graft rejection in the postoperative period. Despite one or more episodes of graft reaction, some grafts would finally clear after administration of sub-conjunctival and systemic steroid treatment. Those corneas were considered accepted. Corneal grafts that had clinical signs of graft reaction and had never cleared were considered rejected or failed.

Statistical analysis

Descriptive statistics was used to calculate median values with ranges and lower and upper quartiles. Kruskal-Wallis median test was used for between-group analysis of variance, with Man-Whitney test for post hoc analysis and between-group comparison. Kaplan-Meier curves were generated for the analysis of time period to corneal graft rejection. Differences were considered significant at $P<0.05$. The software used for data analysis was Statistica for Windows 5.0 (StatSoft Inc., Tulsa, OK, USA).

Results

Interleukin-1α production

The median production of IL-1α of 5.92 pg/mm^3 (range, 0.48-12.68) in corneas with inflammatory diseases was significantly higher than that in corneas with non-inflammatory diseases (median, 2.47 pg/mm^3; range, 0.13-9.95; $P = 0.005$) and control corneas (median, 0.63 pg/mm^3; range 0.36-1.29; $P<0.001$) (Figure 1). There was also a significant difference in IL-1α production between the control corneas and corneas with non-inflammatory diseases ($P = 0.008$). IL-1α was absent from all patient's sera, which confirmed its local intra-ocular production (Figure 1).

There was higher secretion of IL-1α in corneas with inflammatory corneal diseases, which are at increased clinical risk of graft rejection, than in control corneas or corneas with non-inflammatory diseases (Table 1). This was true for both the results expressed in pg/mm^3, where adjustment for corneal thickness was made, and for the results expressed in pg/mL, where corneal hydratation might influence the final result. The only exception was high IL-1α expression found in corneas with Groenouw dystrophy type II (median, 7.64 pg/mm^3; range, 5.32-9.95), similar to the values found in inflammatory diseases (Table 1).

TNF-α production

Low TNF-α levels were detected only in 5 of 14 cases of vascularized corneal scars with the concentration ranging from 0.00 to 2.37 pg/mm^3 (median, 0.00 pg/mm^3; in 3 of 11 cas-

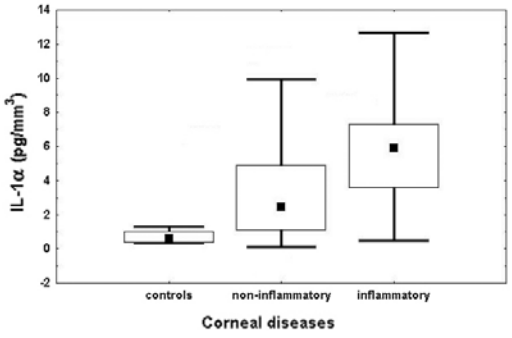

Figure 1. Interleukin-(IL)1α production in diseased human corneas and controls. Human corneas with inflammatory and non-inflammatory diseases were harvested during corneal transplantation, while control corneas were obtained from the eye bank, and cultivated for 24 hours. Concentration of IL-α in corneal supernatant was measured by ELISA, and expressed as median (closed square) with lower and upper quartiles (open square) and minimum and maximum (T lines). The median concentration of IL-1α in inflammatory corneal diseases was significantly increased compared with non-inflammatory ones ($P = 0.005$) and controls ($P<0.001$, post hoc Man-Whitney test). Kruskal-Wallis ANOVA by ranks test confirmed the differences between the tested groups ($P = 0.001$).

Table 1. Production of interleukin (IL)-1a in healthy corneas and corneas with inflammatory and non-inflammatory diseases*

	Corneas							
		inflammatory diseases (n = 24)†			non-inflammatory diseases (n = 23)‡			
Cytokines (median, (range)	control (n = 7)	vascularized corneal scar (n = 14)	rejected graft (n = 6)	corneal ulcer (n = 4)	bullous keratopathy (n = 11)	keratoconus (n = 8)	keratoglobus (n = 2)	dystrophy (n = 2)
IL-1a (pg/mm^3)§	0.63 (0.36-1.29)	6.19 (0.48-12.68)	4.06 (1.52-7.51)	7.51 (5.23-9.10)	4.37 (0.17-7.25)	1.66 (0.13-2.47)	3.61 (3.15-4.06)	7.64 (5.32-9.95)
IL-1a (pg/mL)¶	31.50 (16.00-145.00)	246.64 (21.29-420.68)	208.15 (71.41-331.88)	342.73 (201.16-368.15)	188.87 (7.19-390.20)	72.79 (4.78-99.24)	204.52 (178.60-230.44)	293.815 (204.66-382.97)

*Concentration of each cytokine was measured in corneal supernatant by enzyme-linked immunosorbent assay (ELISA).
†P = 0.005 vs non-inflammatory diseases and P<0.001 vs control corneas (Man-Whitney test).
‡P = 0.008 vs control corneas (Man-Whitney test).
§Results adjusted for corneal thickness.
¶Results expressed per mL supernatant.

es with bullous keratopathy with concentration ranging from 0.00 to 1.50 pg/mm^3 (median, 0.00 pg/mm^3), and in half of the patients with a rejected graft and concentration ranging from 0.00 to 1.14 pg/mm^3 (median, 0.09 pg/mm^3). However, even this small production was a result of local TNF-α secretion because all sera samples were negative for TNF-α.

Association of cytokine concentration in collected corneas and clinical outcome of corneal grafts

Prospective 18-month follow-up of our patients showed that the rejection rate in group with inflammatory diseases was significantly higher than the rate in the group with non-inflammatory conditions ($P = 0.005$).

Graft rejection developed in 11 of 24 recipients with inflammatory corneal diseases who had increased IL-1α production at the time of surgery and in 2 of 23 recipients with non-inflammatory diseases who had low IL-1α concentration at the time of surgery (Table 2). This rejection rate was significantly higher in recipients with inflammatory than in those with non-inflammatory corneal diseases (Fisher exact test, $P = 0.008$).

Exceptions were the patients with corneal dystrophy that had no signs of graft reaction despite high IL-1α production. Kaplan-Meier curves showed that the time-to-graft rejection was significantly shorter and graft rejection was higher in the group of patients with inflammatory diseases (Figure 2). Log-rank test showed

Table 2. Production of pro-inflammatory cytokines interleukin-1alpha (IL-1α) and tumor necrosis factor-alpha (TNF-α) in patients with rejected grafts*

		Pro-inflammatory cytokines (pg/mm^3)	
Patient initials	Diagnosis	IL-1α	TNF-α
Non-inflammatory diseases:			
D. P.	keratoglobus	4.06	0.04
M. M	bullous keratopathy	6.30	0.11
Inflammatory diseases:			
G. D.	rejected graft	4.20	0.16
N. Č.	rejected graft	7.51	0.53
M. B.	rejected graft	5.91	ND*
F. O.	corneal ulcer	6.68	ND
P. B.	corneal ulcer	8.34	ND
F. P.	corneal scar	7.18	1.90
LJ. P.	corneal scar	7.43	ND
I. J.	corneal scar	7.62	1.26
N. N.	corneal scar	5.93	ND
I. P.	corneal scar	2.78	ND
M. P.	corneal scar	12.68	ND

*ND – not detectable.

significant difference between the groups in distribution of time-to-rejection of corneal graft ($P = 0.004$), and Fisher exact test showed significant difference between the groups in frequency of rejection corneal graft ($P = 0.008$).

Discussion

This study showed that significantly more IL-1α was secreted from corneal buttons excised from corneal graft recipients with inflammatory diseases than from those with non-inflammatory diseases or controls. In the group of patients with inflammatory diseases, high concentration of IL-1α in recipient corneas at the time of surgery led to higher corneal graft rejection rate

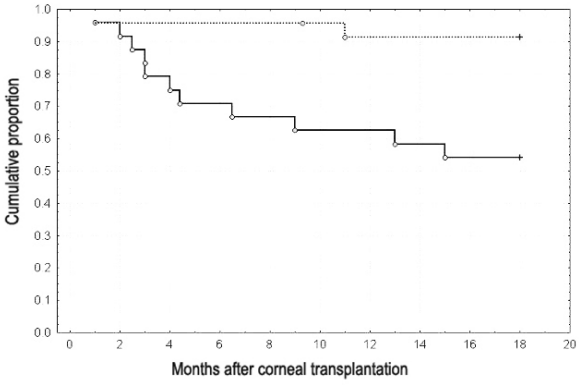

Figure 2. Kaplan-Meier curves showed that time period to graft rejection was shorter and graft rejection was higher in inflammatory group of patients (P = 0.004). Circle – rejected corneas; plus sign – non-rejected corneas; full line – corneas with inflammatory diseases; dotted line – corneas with non-inflammatory diseases.

than in patients with non-inflammatory diseases. These findings suggest that locally produced IL-1α might be partly responsible on a molecular level for high rejection rate in the eyes with inflammatory corneal diseases. We were aware of the fact that the IL-1α concentration in the recipient's graft bed might be different from the concentrations in the extracted central cornea due to different density of immune cells at the corneal periphery. However, it is reasonable to expect that recipients with high IL-1α concentration in the extracted central cornea have even higher amount of IL-1α at the corneal periphery, because the graft bed contains higher amount of antigen-presenting and inflammatory cells then does the central corneal region (20,24), as graft bed is the only source of inflammatory cells from which they migrate into the central cornea.

Since corneal thickness could be increased not only due to the higher number of cells producing the cytokine of interest, but also due to dysfunctional endothelial cells, which results in hydration of the cornea, the results were expressed in pg/mm^3, where adjustment for corneal thickness was made, as well as in pg/mL, where corneal hydratation might influence final result. In both cases, level of IL-1α was significantly higher than that in control corneas. Thus, the detected differences in IL-1α production between the groups were indeed the result of increased cytokine production and not a false result due to higher hydration of a specific corneal sample.

Unexpectedly, patients with Groenouw dystrophy type II, which is a non-inflammatory corneal disease, also had high concentrations of proinflammatory cytokine IL-1α in their corneas at the time of surgery. No signs of graft rejection were recorded during the follow-up period, but there were only two patients with this disease included in the study.

It is well known from clinical experience that patients suffering from inflammatory corneal diseases are at much higher risk of graft rejection; however, mediators of this phenomenon have yet to be determined (17). According to the available data from animal models, IL-1α promotes allograft rejection in the first graft by stimulation of the antigen-presenting cells and induction of corneal neovascularization (29). Further research in our laboratory is under way to determine if patients with increasing amount of corneal neovascularization also have increased quantity of IL-1α in their corneas.

In the eyes that rejected a corneal graft, despite having a low IL-1α concentration comparable to the one measured in eyes with non-inflammatory corneal diseases, graft rejection rate was high. This indicates that in repeated human corneal grafts, other cytokine(s) act as allograft rejection mediator(s).

IL-1α production in non-inflammatory corneal diseases was significantly higher than in controls. This finding implicates that the eyes clinically considered before operation to be "without" any inflammation may in fact secrete proinflammatory cytokine from the corneas. However, graft rejection in these patients was low. This might be explained by the fact that a higher level of IL-1α production is needed to induce graft rejection, or that an antagonist of IL-1α activity is also secreted from the corneas of low-risk patients, thus preventing graft rejection develop-

ment. In any case, preoperative clinical judgment of the "inflammation rate" does not seem to be as accurate as an objective measurement of cytokine concentration at the time of surgery.

Although TNF-α was also shown to be important graft rejection mediator in animal models (4,32), low TNF-α concentration found in this study, even in the eyes with inflammatory corneal diseases, question its role in humans. Low TNF-α production in inflammatory corneal diseases in humans might also be explained by a different timing of its secretion or indicate a need for more sensitive detection method such as mRNA expression by polymerase chain reaction.

We investigated production of IL-1α and TNF-α in diseased human corneas, because these two cytokines play a role in corneal graft rejection in animal model. The analysis of cytokine concentrations in the aqueous humor and cornea at the time of surgery can help not only in prognosis of graft survival and, therefore, proper decision of postoperative treatment for each patient, but also in possible therapy with proinflammatory cytokine antagonists or immunosuppressive cytokine agonists. Previously, we investigated corneal graft prognosis in human recipients with high and low TGF-β_2 concentrations in aqueous humor, and concluded that the loss of immune privilege, ie, low concentration of TGF-β_2 in the anterior chamber, would increase graft rejection rate (15). According to that study, loss of immune privilege might also be explained by increased production of IL-1α in the recipient cornea.

The present study showed that IL-1α and TNF-α were not the only parameters involved in the mediation of rejection reaction as the correlation between grafting failure and the cytokine concentration was not that linear. Other parameters involved in the mediation of rejection have already been identified (34), whereas other still need to be investigated.

One of the limitations of this study was that we quantified IL-1α and TNF-α level by ELISA testing, which may not correspond to the biological activity of these proteins. Therefore, other testing procedures overcoming this limitation should be considered in future studies in the role of this cytokines in corneal grafting.

In conclusion, it has been shown in animal models that pro-inflammatory actions of IL-1α may be abrogated by topical treatment with its antagonist, IL-1 receptor antagonist (Il-1ra). Topical treatment with IL-1ra can suppress corneal neovascularization (35,36) and promote corneal allograft survival in animal eyes (28-31). Our results, although based on a relatively small sample, implicate that IL-1α is closely involved in corneal rejection in human subjects. Therefore, topical IL-1ra treatment may be useful also in human corneal graft recipients, by lowering the risk, or even preventing, corneal allograft rejection.

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References

1 de Vos AF, Klaren VN, Kijlstra A. Expression of multiple cytokines and IL-1RA in the uvea and retina during endotoxin-induced uveitis in the rat. Invest Ophthalmol Vis Sci. 1994;35:3873-83. Medline:7928184

2 Perez-Guijo V, Santos-Lacomba M, Sanchez-Hernandez M, Castro-Villegas Mdel C, Gallardo-Galera JM, Collantes-Estevez E. Tumour necrosis factor-alpha levels in aqueous humour and serum from patients with uveitis: the involvement of HLA-B27. Curr Med Res Opin. 2004;20:155-7. Medline:15006008

3 Yamagami S, Kawashima H, Endo H, Tsuru T, Shibui H, Kagawa Y, et al. Cytokine profiles of aqueous humor and graft in orthotopic mouse corneal transplantation. Transplantation. 1998;66:1504-10. Medline:9869092

4 Zhu S, Dekaris I, Duncker G, Dana MR. Early expression of proinflammatory cytokines interleukin-1 and tumor necrosis factor-alpha after corneal transplantation. J Interferon Cytokine Res. 1999;19:661-9. Medline:10433368

5 Geiger KD, Sarvetnick NE. Ocular production of interferon-gamma and lack of major histocompatibility complex molecules induce immunological changes in the intraocular environment. Ger J Ophthalmol. 1996;5:309-14. Medline:9479510

6 Okamoto S, Streilein JW. Role of inflammatory cytokines in induction of anterior chamber-associated immune deviation. Ocul Immunol Inflamm. 1998;6:1-11. Medline:9798188

7 Balkwill FR, Burke F. The cytokine network. Immunol Today. 1989;10:299-304. Medline:2686679

8 Barker CF, Billingham RE. Adv Immunol. 1977;25:1-54. Medline:345773.

9 Streilein JW, Wilbanks GA, Cousins SW. Immunoregulatory mechanisms of the eye. J Neuroimmunol. 1992;39:185-200. Medline:1644895

10 Niederkorn JY. Immune privilege and immune regulation in the eye. Adv Immunol. 1990;48:191-226. Medline:2190450

11 Streilein JW, Niederkorn JY. Induction of anterior chamber-associated immune deviation requires an intact, functional spleen. J Exp Med. 1981;153:1058-67. Medline:6788883

12 Streilein JW, Cousins SW. Aqueous humor factors and their effect on the immune response in the anterior chamber. Curr Eye Res. 1990;9 Suppl:175-82. Medline:2200643

13 Taylor AW, Streilein JW, Cousins SW. Identification of alpha-melanocyte stimulating hormone as a potential immunosuppressive factor in aqueous humor. Curr Eye Res. 1992;11:1199-206. Medline:1490338

14 Streilein JW, Bradley D, Sano Y, Sonoda Y. Immunosuppressive properties of tissues obtained from eyes with experimentally manipulated corneas. Invest Ophthalmol Vis Sci. 1996;37:413-24. Medline:8603847

15 Dekaris I, Gabric N, Mazuran R, Karaman Z, Mravicic I. Profile of cytokines in aqueous humor from corneal graft recipients. Croat Med J. 2001;42:650-6. Medline:11740849

16 Dana MR, Streilein JW. Loss and restoration of immune privilege in eyes with corneal neovascularization. Invest Ophthalmol Vis Sci. 1996;37:2485-94. Medline:8933765

17 The Collaborative Corneal Transplantation Studies Research Group. The collaborative corneal transplantation studies (CCTS). Effectiveness of histocompatibility matching in high-risk corneal transplantation. Arch Ophthalmol. 1992;110:1392-403. Medline:1417537

18 Streilein JW, Bradley D, Sano Y. Immune privilege in murine eye with abnormal corneas. In: The Association for Research in Vision and Ophthalmology Annual Meeting, Abstracts; 1994 May 1-6; Sarasota, Florida, USA. Invest Ophthalmol Vis Sci. 1994;35:1737.

19 Sano Y, Streilein JW. Effect of suture-induced corneal neovascularization on the ocular immunosuppressive microenvironment. In: Proceedings for the Third International Symposium on Recent Development in the Immunopathology and Intraocular Inflammation. Miami, Florida, Oct 10-14, 1993. Reg Immunol. 1994;6:173-6.

20 Dekaris I, Zhu SN, Dana MR. TNF-alpha regulates corneal Langerhans cell migration. J Immunol. 1999;162:4235-9. Medline:10201952

21 Sano I, Takahashi T, Koji T, Udono H, Yui K, Ayabe H. Prolonged survival of rat cardiac allograft with pro-inflammatory cytokine inhibitor. J Heart Lung Transplant. 2001;20:583-9. Medline:11343986

22 Roayaie S, Sheiner PA, Emre S, Guy S, Schwartz ME, Boros P, et al. Cytokine profiles in early rejection following OKT3 treatment in liver transplant patients. Mediators Inflamm. 2000;9:141-6. Medline:11132770

23 Torres PF, De Vos AF, van der Gaag R, Martins B, Kijlstra A. Cytokine mRNA expression during experimental corneal allograft rejection. Exp Eye Res. 1996;63:453-61. Medline:8944552

24 Larkin DF, Calder VL, Lightman SL. Identification and characterization of cells infiltrating the graft and aqueous humour in rat corneal allograft rejection. Clin Exp Immunol. 1997;107:381-91. Medline:9030879

25 Arancibia-Carcamo CV, Osawa H, Arnett HA, Haskova Z, George AJ, Ono SJ, et al. A CIITA-independent pathway that promotes expression of endogenous rather than exogenous peptides in immune-privileged sites. Eur J Immunol. 2004;34:471-80. Medline:14768052

26 King WJ, Comer RM, Hudde T, Larkin DF, George AJ. Cytokine and chemokine expression kinetics after corneal transplantation. Transplantation. 2000;70:1225-33. Medline:11063345

27 Yamada J, Dana MR, Sotozono C, Kinoshita S. Local suppression of IL-1 by receptor antagonist in the rat model of corneal alkali injury. Exp Eye Res. 2003;76:161-7. Medline:12565803

28 Jie Y, Zhang WH, Pan ZQ, Wu YY, Wang Y. Interleukin-1 receptor antagonist eye drops promoting high-risk corneal allografts survival in rats. Chin Med J (Engl). 2004;117:711-6. Medline:15161539

29 Dana MR, Yamada J, Streilein JW. Topical interleukin 1 receptor antagonist promotes corneal transplant survival. Transplantation. 1997;63:1501-7. Medline:9175817

30 Yamada J, Zhu SN, Streilein JW, Dana MR. Interleukin-1 receptor antagonist therapy and induction of anterior chamber-associated immune deviation-type tolerance after corneal transplantation. Invest Ophthalmol Vis Sci. 2000;41:4203-8. Medline:11095616

31 Yamada J, Dana MR, Zhu SN, Alard P, Streilein JW. Interleukin 1 receptor antagonist suppresses allosensitization in corneal transplantation. Arch Ophthalmol. 1998;116:1351-7. Medline:9790635

32 Qian Y, Dekaris I, Yamagami S, Dana MR. Topical soluble tumor necrosis factor receptor type I suppresses ocular chemokine gene expression and rejection of allogeneic corneal transplants. Arch Ophthalmol. 2000;118:1666-71. Medline:11115261

33 World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Available at: *http://www.wma.net/e/policy/b3.htm*. Accessed: November 23, 2005.

34 Williams KA, White MA, Ash JK, Coster DJ. Leukocytes in the graft bed associated with corneal graft failure. Analysis by immunohistology and actuarial graft survival. Ophthalmology. 1989;96:38-44. Medline:2465520

35 Dana MR, Zhu SN, Yamada J. Topical modulation of interleukin-1 activity in corneal neovascularization. Cornea. 1998;17:403-9. Medline:9676913

36 Coxon A, Bolon B, Estrada J, Kaufman S, Scully S, Rattan A, et al. Inhibition of interleukin-1 but not tumor necrosis factor suppresses neovascularization in rat models of corneal angiogenesis and adjuvant arthritis. Arthritis Rheum. 2002;46:2604-12. Medline:12384918