

# Interleukin-1 $\alpha$ Production in Human Corneal Scars

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## ABSTRACT

We studied IL-1 $\alpha$  level in corneal scars with /without neo-vascularization. A total of 27 patients underwent grafting for corneal scar. Recipients were grouped according to number of vascularized quadrants (0 to IV/IV): none (n=12), one (n=5), two (n=4) and four (n=6). Recipient corneas were collected during surgery and IL-1 $\alpha$  measured by immunoassay. Controls were donor corneas unsuitable for transplantation. Graft rejection rate was calculated for each group. Mean IL-1 $\alpha$  concentration in corneal scars was  $6\pm 3.93$  pg/mm<sup>3</sup>; significantly higher as compared to controls ( $1.25\pm 2.03$  pg/mm<sup>3</sup>). IL-1 $\alpha$  correlated well with amount of blood vessels, except in IV/IV scars:  $5.17\pm 3.65$  pg/mm<sup>3</sup> for 0/IV;  $8.02\pm 2.51$  pg/mm<sup>3</sup> for I/IV;  $8.27\pm 3.62$  pg/mm<sup>3</sup> for II/IV and  $4.47\pm 5.03$  pg/mm<sup>3</sup> for IV/IV corneal scars. Vascularization of corneal scar is associated with increased IL-1 $\alpha$  level (in all but highly vascularized scars), indicating that IL-1 $\alpha$  promotes early stages of vascularization. Graft rejection rate increases in patients with higher vascularization, independently of IL-1 $\alpha$  level.

**Key words:** Interleukin-1 $\alpha$ , scars, cornea, humans

## Introduction

Corneal transplantation is a highly successful transplantation with a success rate of over 90% in patients suffering from non-inflammatory corneal diseases. However, in patients undergoing corneal grafting due to neovascularized corneal scars success rate decreases to 50–60%. Studies in animal model have established that neovascularization of the cornea leads to significant inflammatory cell infiltration and increased rejection rate if allograft is placed in such a recipient bed. These studies have also shown that graft rejection is mediated primarily by CD4+ T cells of the T helper 1 (Th1) phenotype and that presence of neovascularization supports migration of inflammatory cells<sup>1</sup>.

Several mediators have been implicated to induce migration of inflammatory cells and thus promote corneal allograft rejection, for example IL-1 $\alpha$  and TNF- $\alpha$ . These cytokines stimulate migration of antigen-presenting cells toward central cornea (where the allograft is placed), increase expression of adhesion and co-stimulatory molecules on corneal cells and promote neovascularization of the cornea<sup>2,3</sup>. Presence of vessels in the corneal tissue enables migration of antigen presenting and other inflammatory cells toward corneal allograft and

consequent graft rejection<sup>4,5</sup>. It has been shown in animal model that increased amounts of IL-1 alpha and TNF-alpha can be found in supernatants from grafted corneas early after allotransplantation<sup>6</sup>.

From the clinical experience with human grafts it is well known that a success rate of corneal graft decreases if allograft is placed in vascularized recipient bed<sup>7</sup>. Therefore, patients with corneal neovascularization are usually considered as »high-risk« cases with a significant risk for allograft rejection development. Several degrees of corneal neovascularization might be present in a recipient bed prior to surgery. Therefore, patients are usually grouped according to the number of vascularized corneal quadrants (one to all four quadrants). It is still unclear whether IL-1 $\alpha$  promotes ingrowth of new vessels after corneal transplantation in humans, enabling in this way free migration of inflammatory cells into and out of the grafted tissue. In our study we have examined IL-1 $\alpha$  expression in human corneal tissue with increasing amount of vascularization at the time of corneal grafting and followed these patients to establish rejection rate.

## Subjects and Methods

Human tissue used in these experiments was obtained and managed in accordance with the provisions of the Declaration of Helsinki. Corneal tissue buttons were obtained from 27 patients suffering from either non-vascularized or vascularized corneal scars. Control corneas were obtained from healthy donors (corneas that were unsuitable for transplantation due to inadequate endothelial cells). Preoperatively patients were divided into two groups according to the presence of vascularization in their corneas: non-vascularized (0/IV; n=12) and vascularized corneal scars (I/IV till IV/IV; n=15). The latter group was subdivided according to number of vascularized corneal quadrants: A) one quadrant (I/IV; n=5), B) two quadrants (II/IV; n=4), C) four quadrants (IV/IV; n=6). Patients were operated at our setting over the period of 2002 till 2004. Informed consent was obtained from all patients after explanation of the nature of study. The study included 19 male and 8 female patients (aged 20–70 years). Serum samples were collected at the time of surgery to measure the amount of investigated cytokine in a peripheral blood.

### Tissue collection

Thickness of each diseased and control cornea was measured by ultrasound pachimetry (Alcon Surgical 8700) prior to surgery. Corneal samples were collected during corneal transplantation by trephination of a diseased corneal button from a graft recipient. Diameter of each trephined corneal sample was recorded. Corneal buttons were immediately cultivated for 24 hours at 37 °C in 0.5 ml of corneal storage media (CorneaPrep, EuroBio, France). After cultivation, corneas and there supernatant were frozen and kept at –21 °C until cytokine detection.

### Cytokine quantization

Concentrations of IL-1 $\alpha$  in a supernatant of cultivated corneas, and in sera were determined by using a commercial ELISA kits (Quantikine, R&D Systems, USA), according to the manufacturer's instructions. Each sample was tested by ELISA twice. Cytokine concentration was calculated and adjusted for each cultivated cornea according to its volume by the following formula:  $r^2 \times \pi \times h$  ( $r$  – corneal diameter,  $h$  – corneal thickness,  $\pi = 3.14$ ), and expressed per mm<sup>3</sup> of tissue.

### Association of cytokine level and clinical signs of graft reaction

Corneal graft recipients that donated their diseased corneas for this experiment were followed up for at least one year after surgery and each clinical sign of graft reaction was recorded. Postoperatively, patients with non-vascularized or poorly vascularized corneal scars (groups A and B) received only topical steroid treatment, while those with highly vascularized scars (group C) received topical and systemic steroid treatment. Clinical signs of corneal graft reaction were following: (a) epithelial re-

jection – 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 oedema and haze in a previously clear graft; (d) presence of the endothelial rejection line or diffuse keratic precipitates on endothelium. Diagnose 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 corneas on the day of surgery were associated with the presence of mentioned signs 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, which had clinical signs of graft reaction and have never cleared, were considered rejected.

### Statistical analysis

To test difference between cytokine production in investigated groups, Student *t*-test was used. The normality of distributions was tested by Kolmogorov-Smirnov test.  $p < 0.05$  was considered to indicate statistical significance.

## Results

### IL-1 $\alpha$ production

The concentration of IL-1 $\alpha$  secreted from control corneas was  $1.25 \pm 2.03$  pg/mm<sup>3</sup>. Significantly higher IL-1 $\alpha$  production was found in all patients suffering from both vascularized and non-vascularized corneal scar ( $6.68 \pm 4.14$  and  $5.18 \pm 3.66$ ;  $p < 0.005$ ). (Figure 1). IL-1 $\alpha$  was absent in all patient's sera, confirming its local intra-ocular production.

Mean IL-1 $\alpha$  production correlated well with the amount of blood vessels in the cornea in all groups except highly vascularized scars:  $5.17 \pm 3.65$  pg/mm<sup>3</sup> for 0/IV;

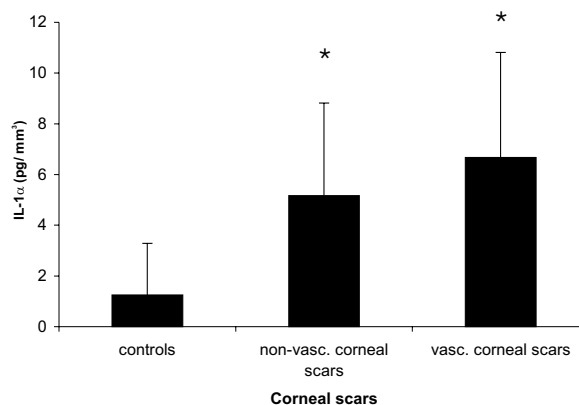


Fig. 1. IL-1 $\alpha$  production in vascularized, non-vascularized corneal scars and controls. Concentration of IL-1 $\alpha$  is expressed as mean  $\pm$  SD. \* Mean concentration of IL-1 $\alpha$  in both non-vascularized and vascularized corneal scars was significantly elevated compared with controls ( $p < 0.005$ ).

8.02 $\pm$ 2.51 pg/mm<sup>3</sup> for I/IV; 8.27 $\pm$ 3.62 pg/mm<sup>3</sup> for II/IV and 4.47 $\pm$ 5.03 pg/mm<sup>3</sup> for IV/IV corneal scars. (Table 1.)

**TABLE 1**  
IL-1 $\alpha$  PRODUCTION BY EACH EVALUATED GROUP OF PATIENTS ACCORDING TO CORNEAL VASCULARIZATION

Quadrants of corneal vascularization	Number of patients	IL-1 alpha pg/mm <sup>3</sup> (mean $\pm$ SD)
none	12	5.17 $\pm$ 3.65
I	5	8.02 $\pm$ 2.51
II	4	8.27 $\pm$ 3.62
IV	6	4.47 $\pm$ 5.03

#### *Association of cytokine concentration in collected corneas with clinical outcome of corneal grafts*

Prospective follow-up (at least one year) of our patients have shown that the corneal graft rejection rate did not correlate well with the amount of IL-1 $\alpha$  production at the time of surgery. Namely, higher levels of IL-1 $\alpha$  in a particular patient at the time of surgery did not necessarily lead to graft rejection (see Table 2). However, prospectively higher rejection rates were recorded with the increased number of vascularized corneal quadrants in a graft recipient (Table 2) Graft rejection rate was highest (50%) in recipients with 4 vascularized quadrants and significantly decreased in patients with two (25%) and one (20%) vascularized quadrants. In patients with non-vascularized corneal scars 2 out of 12 (16%) of patients ended-up with graft failure. Since no signs of graft rejection were recorded in one of these two cases, graft failure was associated with patients' dry eye syndrome and this case was not calculated as graft rejection. Typical signs of graft rejection were recorded in one patient from non-vascularized group, resulting in graft rejection rate of 8%.

## Discussion

The normal cornea is devoid of blood and lymphatic vessels but can become vascularized secondary to a variety of corneal diseases<sup>8</sup>. Previous studies in animal corneas have shown that interleukin-1 (IL-1) acts as a critical mediator in the early phase of corneal neovascularization (CNV)<sup>9</sup>. Same authors have shown that topical treatment with interleukin-1 receptor antagonist (IL-1ra) can effectively suppress CNV if initiated early after the inflammatory neovascular stimulus<sup>9</sup>. The administration of IL-1 was shown to reduce the influx into the cornea of cells of the innate and adaptive immune response<sup>10</sup>. In addition, the treatment diminished corneal vascular endothelial growth factor levels, resulting in reduced angiogenic response.

Corneal allotransplantation is the most common and successful form of transplantation in humans. This extraordinary success is attributed to various features of

**TABLE 2**  
IL-1 $\alpha$  PRODUCTION AND GRAFT OUTCOME RECORDED FOR EACH PATIENT INCLUDED IN THE STUDY

Patient's initial	Quadrants of corneal vascularisation	IL-1 alpha (pg/mm <sup>3</sup> )	Graft outcome
S.S.	0 / IV	8.32	rejected due to dry eye
A.B.	0 / IV	4.64	clear
S.K.	0 / IV	2.15	rejected
I.Ž.	0 / IV	9.76	clear
V.E.	0 / IV	1.24	clear
S.K.	0 / IV	0.98	clear
I.N.	0 / IV	2.23	clear
B.V.	0 / IV	7.61	clear
V.S.	0 / IV	5.49	clear
Z.K.	0 / IV	0.89	clear
V.G.	0 / IV	7.90	clear
S.P.	0 / IV	10.94	clear
J.Š.	I / IV	9.53	rejected
Z.Š.	I / IV	8.02	clear
S.S.	I / IV	6.72	clear
T.Ž.	I / IV	11.20	clear
J.V.	I / IV	4.67	clear
I.B.	II / IV	8.67	clear
C.G.	II / IV	7.50	clear
A.V.	II / IV	4.07	clear
I.M.	II / IV	12.86	rejected
Ž.V.	IV / IV	0.07	clear
I.M.	IV / IV	10.70	rejected
M.Š.	IV / IV	0.04	clear
J.K.	IV / IV	0.01	rejected
M.J.	IV / IV	9.38	clear
M.M.	IV / IV	6.67	rejected

the normal cornea and anterior segment that together account for their »immune-privileged« status<sup>11</sup>. However, corneal angiogenesis in vascularized high-risk beds provides a route of entry for immune effector cells to the graft, and in such patients, a significant number of corneal grafts fail<sup>7</sup>. Having in mind that IL-1 $\alpha$  promotes corneal vascularization at its early phase, and that this activity can be effectively suppressed by IL-1ra eye-drops<sup>12</sup>, we investigated whether IL-1 is secreted from corneas of human graft recipients with different amount of CNV. Our goal was to determine whether inflammatory corneal neovascularization (CNV) in humans is associated with interleukin-1 (IL-1) activity and if so, to establish whether antiangiogenic strategies (e.g. IL-1ra eye drops) have a potential to improve transplant survival in humans.

Our results, although in a relatively small group of patients, have shown that both non-vascularized and

vascularized corneal scars produce significantly higher amounts of IL-1 $\alpha$  than control corneas. Moreover, increased amount of IL-1 $\alpha$  was detected as the number of vascularized corneal quadrants increased, except for highly (all four quadrants) vascularized grafts. This data suggest that IL-1 may promote »early« phase of CNV in human corneas. Our findings are in accordance with data in animal model, where it has been shown that IL-1 $\alpha$  acts only at the early phase of the CNV<sup>9</sup>. Hence, early targeting of proinflammatory molecules such as IL-1, might be beneficial to counteract CNV formation in human corneas. However, once the CNV is already formed, IL-1 $\alpha$  does not seem to play a crucial role in further immune developments.

Graft survival rate in the examined eyes depended on the amount of CNV prior to surgery, but did not correlate well with the detected amount of IL-1 $\alpha$  at the time of surgery. This, unfortunately, means that measurement of IL-1 $\alpha$  level at the time of surgery can not influence our decision for optimal postoperative treatment in a specific patient (only topical or both topical and systemic steroid treatment!).

However, presence of significantly higher amount of IL-1 $\alpha$  as compared to control corneas, and its increased

level as the CNV progresses, may implicate beneficial effect of IL-1 antagonist in preventing development of CNV, thus indirectly also increasing the chance of corneal graft survival. According to our results, topical administration of IL-1 antagonist should be started in non-vascularized or poorly vascularized corneal scars in order to prevent further CNV. Topical eye drops may be sufficient to achieve this result since IL-1 $\alpha$  in human corneas results from its local (intraocular) production. Namely, all the patients' sera, regardless of the investigated group, were devoid of IL-1 $\alpha$ .

Once the newly formed vessels cover all 4 corneal quadrants, free exit of antigen-presenting cells and antigenic material from the graft to regional lymph nodes is enabled, thus inducing alloimmunization and subsequent graft rejection. Blockage of IL-1 $\alpha$  at this stage would hardly be effective in a prevention of allograft rejection.

### Acknowledgements

This paper was supported by Croatian Ministry of Science, Education and Sport Grant 0129010 – Immunology of the Eye.

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### PRODUKCIJA IL-1 $\alpha$ U PACIJENATA S LEUKOMOM ROŽNICE

#### SAŽETAK

Istraživana je produkcija IL-1  $\alpha$  u rožnicama pacijenata s nevaskeulariziranim i vaskulariziranim leukomom. Transplantacija rožnice učinjena je u 27 pacijenata s leukomom rožnice. Primatelji su podijeljeni u grupe ovisno o broju vaskulariziranih kvadranta (0 do IV/IV): nula (n=12), jedan (n=5), dva (n=4), četiri (n=6). Primateljve rožnice prikupljene su u operaciji I izmjerena produkcija IL-1  $\alpha$  pomoću imuno-testa. Kontrole su bile donorske rožnice nepogodne za transplantaciju. Za svaku istraživanu grupu određena je stopa odbacivanja transplantata. Srednja koncentracija IL-1  $\alpha$  u leukoma rožnice bila je  $6\pm 3.93$  pg/mm<sup>3</sup>; signifikantno viša u odnosu na kontrole ( $1,25\pm 2,03$  pg/mm<sup>3</sup>). Koncentracija IL-1 $\alpha$  korelirala je s količinom krvnih žila rožnice, osim u IV/IV leukoma:  $5,17\pm 3,65$  pg/mm<sup>3</sup> za 0/IV;  $8,02\pm 2,51$  pg/mm<sup>3</sup> za I/IV;  $8,27\pm 3,62$  pg/mm<sup>3</sup> za II/IV and  $4,47\pm 5,03$  pg/mm<sup>3</sup> za IV/IV leukome rožnice. Vaskularizacija leukoma rožnice povezana je s povišenim nivoom IL-1 $\alpha$  (osim u vaskularizacije u sva četiri kvadranta, što ukazuje na ulogu IL-1 $\alpha$  u promociji vaskularizacije. Stopa odbacivanja transplantata raste s porastom vaskularizacije neovisno o nivou IL-1 $\alpha$ .