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CLINICAL INVESTIGATION

Okihiro Nishi
Kayo Nishi
Kaori Wada
Yasukazu Ohmoto

Expression of transforming growth factor (TGF)-α, TGF-β_2 and interleukin 8 messenger RNA in postsurgical and cultured lens epithelial cells obtained from patients with senile cataracts

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O. Nishi (✉) · K. Nishi
Nishi Eye Hospital,
4-14-26, Nakamichi, Higashinari-ku,
Osaka 537-0025, Japan
e-mail okihiro@nishi-ganka.or.jp
Tel. +81-6-6981-1132
Fax +81-6-6981-5630

K. Wada · Y. Ohmoto
Ohtsuka Pharmaceutical,
Research Center,
463-10, Kaga Suno, Kawauchi-cho,
Tokushima 777-0130, Japan

Abstract ● Background: Testing our hypothesis that residual lens epithelial cells (LEC) participate in the pseudophakic inflammation by producing cytokines, prostaglandins (PG) or both, we detected interleukin-1 (IL-1), IL-6, b-FGF and PGE_2 in the incubation medium of cultured LEC. This paper describes our subsequent work on the expression of TGF-α, TGF-β_2, IL-4 and IL-8 mRNA in postsurgical and cultured LEC. ● Methods: The anterior lens capsule with attached LEC was obtained by anterior capsulotomy during cataract surgery and cultured. Specimens in serum-free medium immediately after surgery or those in serum-added medium after 2 weeks of culture were used. Reverse transcriptase–polymerase chain reaction (PCR) and electrophoresis were used to detect mRNA coding for TGF-α, TGF-β_2, IL-4 and IL-8 in human cataract LEC. ● Results: Electrophoresis of the PCR products showed that appropriately sized amplification products were specific for TGF-α and TGF-β_2 in the specimens immediately after surgery and after culturing, and IL-8 in the cultured LEC. IL-4 was not detected in either group of specimens. ● Conclusions: Human cataract LEC synthesize TGF-α and TGF-β_2 mRNA in situ and after culturing, and the cultured LEC also synthesize IL-8 mRNA. These cytokines may be synthesized by LEC in vitro and play an important role in an autocrine or paracrine manner in the proliferative process of LEC after cataract surgery, which can cause inflammation and aftercataract.

Introduction

Some clinical and experimental studies suggest that residual lens epithelial cells (LEC) participate in early postoperative inflammation, including fibrin reaction after intraocular lens (IOL) implantation [21, 24, 36]. In our previous study [24], aqueous flare intensity (measured by a laser flare cell meter) decreased from the initial peak mainly due to surgical trauma then increased again to a second peak when residual LEC came into contact with the IOL and began to undergo fibrous proliferation 6–14 days after surgery. The second spike was evidence that the blood-aqueous barrier had been disrupted again. In contrast, in the eyes from which residual LEC had been removed by ultrasound aspiration, neither such a second flare peak nor fibrous proliferation of residual LEC was noted. We hypothesized that residual LEC during proliferation synthesize prostaglandin E_2 (PGE_2) and cytokines such as interleukin-1 (IL-1), basic fibroblast growth factor (b-FGF) and transforming growth factor-β (TGF-β). We also assumed that these mediators are responsible for the renewed break down of the blood-aqueous barrier. To test this hypothesis, we sought PGE_2, IL-1α, IL-6, b-FGF and TGF-β in the incubation media of cultured human LEC. PGE2, IL-1α, IL-6 and b-FGF were detected by enzyme-linked immunoabsorbent assay (ELISA) [25, 26]. Since TGF-β was not detectable by ELISA, the expression of TGF-α, TGF-β_2, IL-4 and IL-8 mRNA by LEC was studied here by reverse transcriptase–polymerase chain reaction (RT-PCR).

Materials and methods

Culture of human cataract LEC

LEC of human cataracts were cultured as previously de<~>scribed [26]. Briefly, a circular piece of the anterior capsule with LEC attached was obtained by capsulotomy during cataract surgery and cultured directly without dispersion of the cells. After circular capsulorhexis, the piece of capsule, about 5 mm in diameter, was touched with an irrigation/aspiration tip and withdrawn from the eye by aspiration. The capsular specimen was held with a fine forceps and thoroughly washed with irrigating solution. Each piece of anterior capsule was immediately placed into a vessel containing ISOGEN (Nippon Gene, Toyama) or into the well of a 48-well, multiwell plate containing 0.3 ml of Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS), penicillin G at 100 U/ml, and streptomycin sulfate at 100 μg/ml and then cultured in 100% humidity at 37°C in a 5% CO_2 atmosphere. The medium was changed once a week.

All specimens were obtained from non-diabetic patients with senile cataracts. The age of the patients ranged from 55 to 76 years with a mean of 68.5±6.8 years (n=200).

Detection of cytokine mRNA

Tissue preparation and extraction of mRNA

After 2 weeks of culture in the well, the specimens were subconfluent and used for the study. After removing the incubation medium from each well, cells were washed three times with 0.5 ml calcium- and magnesium-free phosphate-buffered saline (PBS). Total mRNA was extracted from the cells immediately after surgery and from the cultured cells using Isogen (Nippon Gene, Toyama) according to the manual provided with the kit.

Five pieces of the anterior capsule and five cell cultures were used for the detection of each cytokine mRNA immediately after surgery and after culturing, respectively. Four experiments were performed for each cytokine in each group.

Synthesis of cDNA

To obtain complemetary DNA (cDNA), 10 μl of the lysates containing total mRNA was mixed with RT, and the reverse transcription reaction was performed according to the manufacturer's instructions.

Amplification of cDNA by PCR

The sequences of the PCR primers specified for β-actin, TGF-α, TGF-β_2, IL-4 and IL-8 from 5' to 3' are shown in Table 1.

Ten-microliter aliquots of the RT product lysates were used for PCR with the following temperature profiles: initial heating at 94°C for 3 min, then 33 cycles of 0.5 min at 94°C, 2 min annealing at 60°C, and 3 min extension at 72°C.

Electrophoresis of PCR products

Agarose gel (1.5 % gradient) electrophoresis with 10 μl of PCR products, Tris-glycine buffer and 3 μl of dye-glycerol was performed in an electrophoresis apparatus (M.S. Instruments, Tokyo) at 100–300 v and 45 mA for 1 h. Phi X 174/*Hae*III markers (Toyobo, Osaka) were used as molecular size standards: fragment sizes were 1353, 1078, 872, 603, 310, 281, 234, 194, 118 and 72 bp.

Table 1 Polymerase chain reaction primers

Cytokines	Target length	Upstream	Downstream	Sequence location			
β-Actin	218	TACATGGCTGGGGTGTTGAA	AAGAGAGGCATCCTCACCCT	1 222	CAT	1761 bp	
				AAG→ 439			
TGF-α	297	GGCCTGCTTCTTCTGGCTGGCA	ATGGTCCCCTCGGCTGGACAG	1 35	←CGG	886 bp	
				ATG→ 331			
TGF-β_2	503	CAGCATCAGTTACATCGAAGGAGAGCCATTCG	GATTTCCATCTCTACAAGACCACGAGGGACTTGC	1 668	←GAC	2445 bp	
				GAT→ 1170			
IL-4	344	ACGTACTCTGGTTGGCTTCCTTCACAGGACAG	CGGCAACTTTGACCACGGACACAAGTGCGATA	1 117	←GCA	6186 bp	
				CGG→ 460			
IL-8	289	TCTCAGCCCTCTTCAAAAACTTCTC	ATGACTTCCAAGCTGGCCGTGGCT	1	←TCT	297 bp	
				ATG→ 289			

Nucleic acid sequence of PCR products

To confirm that each PCR product was identical with cDNA obtained from mRNA for the corresponding cytokine, the amplified PCR products separated by electrophoresis were cut from agarose gels, cloned into the pT blue T-Vector (Novagen) and sequenced (Thermo Sequence cycle sequencing kit, Amersham) according to the manufacturers' protocols.

Results

Specific electrophoresis bands corre<~>sponding to the base pairs for β-actin (Fig. 1a), TGF-α and, TGF-β_2 according to the Phi X 174/*Hae*III markers in the specimens were always detected immediately after surgery (Fig. 1b) and after culturing (Fig. 2). IL-8 was found only in the cultured specimens (Figs. 1a, 2b). IL-4 was never detected. Nucleic acid sequencing demonstrated that the amplification product of the expected size was identical to the known sequence for β-actin, TGF-α, TGF-β_2 or IL-8.

Discussion

Cytokines are synthesized and secreted peptides or glycoproteins that act at concentrations of 10^{-10}–10^{-15} mol/l to stimulate target cell function [30]. These low concentration levels might have hindered the detection of TGF-β in our previous research. Another reason may be that calf serum used for culturing contained some proteins that are similar in amino acid sequence to human TGF-β, and therefore the monoclonal antibody technique was not sufficient for the detection. RT-PCR was therefore employed to demonstrate the corresponding cytokine mRNA. In the present study, IL-4 and IL-8 were additionally sought, since these cytokines are known to play an important role in inflammation.

The detection of TGF-α and TGF-β_2 mRNA in the specimens immediately after surgery suggests that these cytokines are produced and released in situ by human cataractous LEC. However, whether mRNA for these cytokines is expressed specifically by cataractous LEC or inherently by LEC remains to be clarified. Lens epithelial cells from non-cataractous lenses should be studied. LEC from an eye bank eye were used in one experiment, and TGF mRNA was detected in the specimens before culturing. However, this eye bank eye is of limited value, because the presence of cataract could not be excluded. The use of fetal or newborn lenses may be appropriate, because they are free from senile cataracts.

TGF-α is a multifunctional cell-regulating protein with a wide spectrum of effects on cell growth and differentiation and has been demonstrated to play a significant role in wound healing [10, 31]. TGF-α was reportedly found in the normal and affected tissues of the eye,

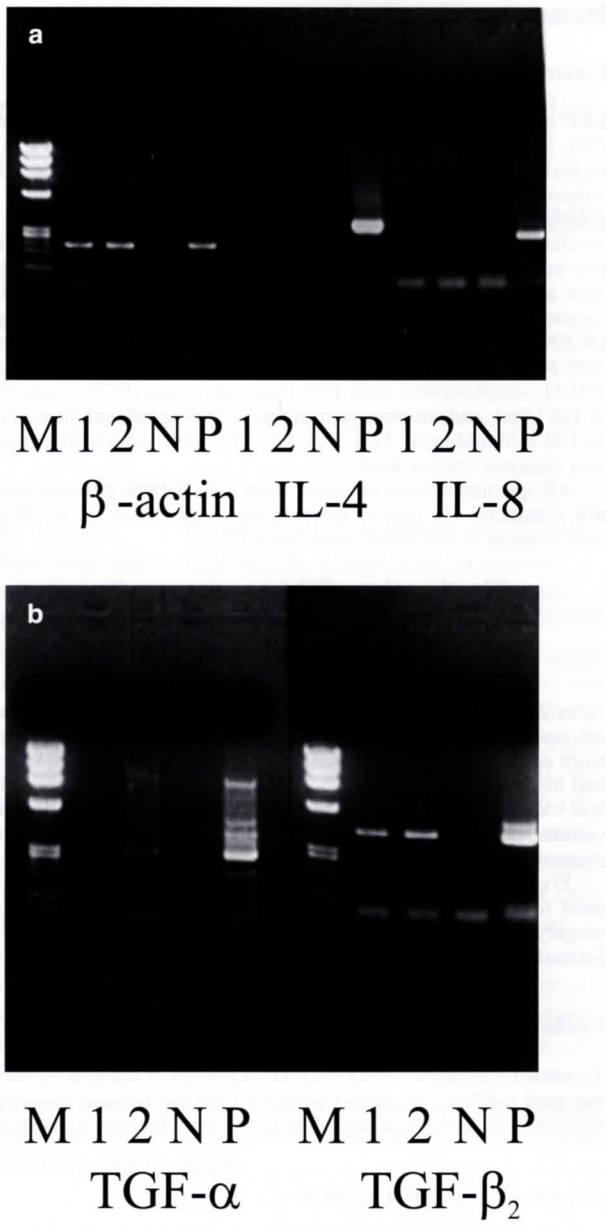

Fig. 1a, b PCR products from the specimens of lens epithelial cells immediately after surgery. *M* Phi X 174 DNA/*Hae*III standard size markers with their lengths in base pairs; *N*, *P* negative and positive controls, respectively; *1*, *2* specimen numbers. Beta-actin, TGF-α and TGF-β_2 were detected, while IL-4 and IL-8 were not

mainly in the retina [10, 31, 32, 35, 39]. TGF-α may be involved in the proliferation and differentiation of lens epithelial cells under physiological conditions and after cataract surgery.

In vitro, TGF-β generally [2] stimulates the proliferation, transformation and differentiation of cells of mesenchymal origin, which increase extracellular matrix components, but inhibits cells of epithelial origin. TGF-β

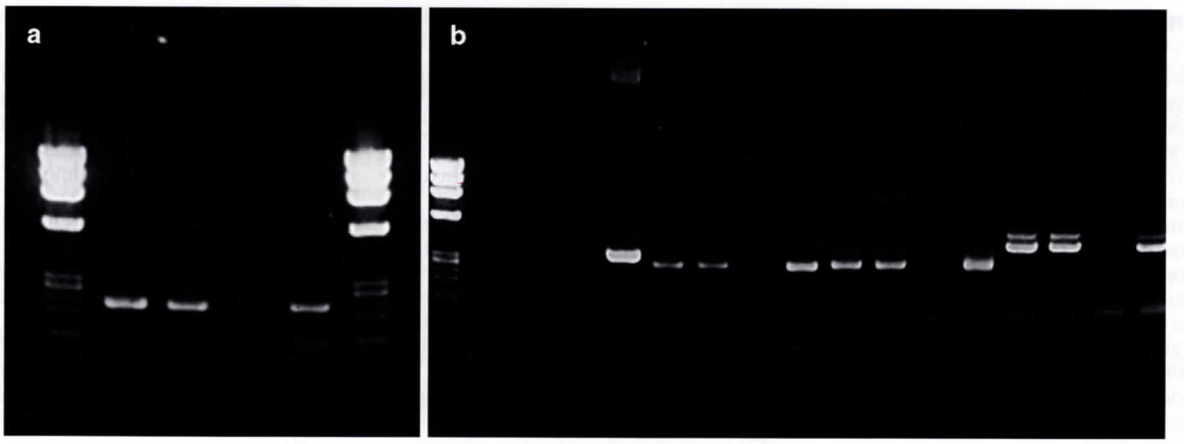

Fig. 2a, b PCR products from the specimens of cultured lens epithelial cells. Beta-actin, TGF-α, TGF-β_2 and IL-8 were detected, while IL-4 was not

has been detected in various normal and inflamed tissues of the eye, and plays an important role in the wound healing process or pathogenesis of various diseases in cornea, lens, uvea and retina [1, 14–16, 22, 28, 34, 38]. TGF-β induces activation and myofibroblast transformation of corneal keratocytes [15]. Topical application of blocking antibodies to TGF-β inhibits corneal fibrosis and haze [15, 22]. TGF-β downregulates proliferation of cultured LEC, but upregulates collagen synthesis by LEC [18, 27]. TGF-β upregulates PGE_2 synthesis by human LEC. TGF-β increases collagen synthesis by retinal pigment epithelial cells and is suggested to effect the contraction of the proliferative membrane in proliferative vitreoretinopathy (PVR) [12, 13]. In vitrectomy combined with IOL implantation in which the nucleus and posterior capsule are removed through pars plana and the IOL is implanted on the remaining anterior lens capsule [4], the remaining LEC underneath the anterior capsule may produce TGF-β and PGE_2 and may worsen PVR due to the lacking posterior capsule. Human LEC produce α-smooth muscle actin (α-SMA) [33], and in bovine LEC, TGF-β_2 increases collagen gel contraction and α-SMA expression [17]. Thus, TGF-β_2 may modulate fibrous proliferation of LEC and fibrous opacification with the contraction of the capsular fibrosis after cataract surgery.

IL-8 mRNA was not detected in the specimens immediately after surgery but was detected in the cultured LEC, while the LEC proliferated and underwent fibroblast-like cell changes in the well. This suggests that the cytokine is produced in vivo by residual LEC first when the LEC undergo fibrous proliferation after IOL implantation. However, whether the expression of mRNA of this cytokine in vitro is an FCS-induced phenomenon remains to be clarified. A study using specimens cultured in serum-free medium should be performed. IL-8 is a cytokine with neutrophil-chemotactic and -activating properties [3, 8] and is known to be stimulated by IL-1 [9]. IL-8 is known to be produced by human retinal pigment epithelial cells [9] and human corneal cells [6, 9]. It was also detected in vitreous fluid samples from patients with uveitis [7]. IL-8-specific mRNA was found in human corneal keratocytes infect<~>ed by herpes simplex virus [29]. Intravitreal injection of IL-8 causes acute inflammation [11]. However, further studies on the effect of IL-8 on human LEC are needed.

IL-4 stimulates activation of T-cells in the pathogenesis of immunological process. Little is known about IL-4 regarding ocular pathophysiology, but it is thought to play a role in the immunopathogenesis of herpetic stromal keratitis [37] and interstitial keratitis by *Onchocerca volvulus* [5]. It also plays a role in allergic conjunctivitis [19]. The relevance of the lack of IL-4 mRNA in LEC needs further study.

IL-1 and IL-6 were detected in the aqueous humor after IOL implantation in rabbits and humans, respectively [20, 40]. b-FGF and TGF-β were also detected in the aqueous humor in rabbits after IOL implantation [23]. This suggests that the cytokines detected in our previous and present studies are produced in vivo as well, and residual LEC may be a source of the cytokines detected in the aqueous humor after IOL implantation.

In summary, IL-8, TGF-α and TGF-β, as well as IL-1, IL-6 and b-FGF, may be produced in vivo by LEC and modulate in an autocrine or paracrine manner the proliferative process of LEC after cataract surgery, which then could cause inflammation and aftercataract.

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