

Effect of the cytokines on the prostaglandin E₂ synthesis by lens epithelial cells of human cataracts

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Abstract

Background—Lens epithelial cells (LECs) derived from human cataracts have been reported to produce various cytokines and prostaglandin E₂ (PGE₂) in culture. The effects of IL-1, TGF-β, and b-FGF on the PGE₂ synthesis by LECs have been studied.

Methods—A circular piece of the anterior capsule with attached LECs was obtained by capsulotomy during cataract surgery and cultured. The primary, almost confluent, cultures were used for the study. The PGE₂ concentration of the culture media for 24 h was measured after the addition of recombinant human IL-1 α, TGF-β 2, or b-FGF at various concentrations. The PGE₂ concentration was also measured in the media to which each cytokine and rabbit polyclonal anti-human antibodies against the corresponding cytokine had been added.

Results—The PGE₂ concentration of the culture media after addition of IL-1 α at the concentration of 100 or 500 pg/ml (1765 (768) and 3071 (1121) pg/10⁴ cells) or TGF-β 2 at the concentration of 10 or 100 ng/ml (689 (264) and 750 (189) pg/10⁴ cells) was significantly increased compared with that in the controls (67 (20) pg/10⁴ cells). These effects were suppressed by the corresponding anti-cytokine antibodies. Basic FGF and anti-human b-FGF showed no significant effect on the PGE₂ concentration. IL-1 and TGF-β increased but b-FGF did not affect the PGE₂ synthesis by LECs in culture.

Conclusion—IL-1 and TGF-β may participate in postoperative inflammation after cataract surgery by increasing PGE₂ synthesis by residual LECs.

(*Br J Ophthalmol* 1995; 79: 934-938)

We previously reported that residual lens epithelial cells (LECs) participate in postoperative inflammation¹ including fibrin reaction,² a fibrinous, non-infectious inflammation seen 4 to 10 days after intraocular lens (IOL) implantation. The aqueous flare intensity, which was decreasing after the initial peak

owing mainly to surgical trauma, increased again forming a second peak, when the LECs came in contact with the IOL and began to undergo fibrous proliferation. Since this second flare peak was not seen when residual LECs were removed, this flare spike – that is, the renewed breakdown of the blood-aqueous barrier, was thought to be caused by residual LECs. Thus, residual LECs can disrupt the blood-aqueous barrier after IOL implantation, enabling them to participate in the production of postoperative inflammation after cataract surgery. Some reports have corroborated this finding. The prostaglandin E₂ (PGE₂) concentration in the aqueous humour has been found to be significantly higher in eyes with IOL implanted in the capsular bag than in those without it.³ The permeability across the blood-aqueous barrier to fluorescein was found to be significantly higher in the in the bag fixations than in the out of the bag fixations.⁴ In these reports, the significant increase in blood-aqueous barrier disruption was attributed to proliferated residual LECs.

We have proposed the hypothesis⁵ that residual LECs synthesise cytokines and PGE₂, which may be responsible for this disruption of the blood-aqueous barrier. We cultured the LECs of human cataracts and detected interleukin-1 (IL-1),⁵ IL-6,⁶ IL-8, transforming growth factor (TGF)-β 1 (unpublished data), basic fibroblast growth factor (b-FGF),⁷ and PGE₂⁵ in the incubation medium.

Cytokines function as intercellular signals that regulate locally in an autocrine or paracrine manner and, at times, systemically, inflammatory host response.⁸ The present study describes our subsequent research performed to ascertain the effects of IL-1, TGF-β, and b-FGF on PGE₂ synthesis by human cataract LECs in culture.

Materials and methods

To determine the effects of each substance on PGE₂ synthesis, we measured the PGE₂ concentration in primary cultures of human cataract LECs to which recombinant human IL-1 α, TGF-β 2, or b-FGF was added at various concentrations and that in cultures in which a cytokine and its neutralising antibody were added.

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Accepted for publication
24 May 1995

PRIMARY CULTURE OF HUMAN CATARACT LECs
Human cataract LECs were cultured as previously described.⁵ Briefly, a circular piece of the anterior capsule with LECs attached was obtained by capsulotomy during cataract surgery and cultured directly without dispersion of the cells. After circular capsulorhexis, the section of anterior capsule, about 5 mm in diameter, was touched with an irrigation/aspiration tip and withdrawn from the eye by aspiration. Each section was held with fine forceps and washed thoroughly with irrigating solution, then placed immediately into a well of a 48 well, multiwell plate containing 0.5 ml of Eagle's minimum essential medium (MEM) containing 10% fetal calf serum, penicillin G at 100 U/ml, and streptomycin sulphate at 100 µg/ml, and cultured in 100% humidity at 37°C in 5% carbon dioxide atmosphere. The culture medium was replaced once a week.

PGE₂ CONCENTRATION IN THE CULTURE MEDIUM

The cell cultures that became almost confluent after 3 to 4 weeks were used for the study.

PGE₂ concentration in the culture to which IL-1 α, TGF-β 2, or b-FGF was added

An aliquot of the culture medium was replaced with 0.5 ml of culture medium containing human recombinant IL-1 α (500 ng/ml, Genzyme, Cambridge, MA, USA) at the concentrations of 10 pg/ml (n=5), 100 pg/ml (n=5), or 500 pg/ml (n=5), TGF-β 2 (1 mg/ml, Austral Biologicals, San Ramon, CA, USA) at 1 ng/ml (n=5), 10 ng/ml (n=6), or 100 ng/ml (n=5) or b-FGF (1 mg/ml, UBI, Lake Placid, NY, USA) at 1 ng/ml (n=5), 10 ng/ml (n=5), or 100 ng/ml (n=6). The PGE₂ concentration in cultures with untreated medium served as the controls (n=16).

PGE₂ concentration in the culture to which the rabbit polyclonal anti-human cytokine antibody was added to neutralise the cytokine added

An aliquot of culture medium was replaced with 0.5 ml of culture medium containing recombinant human IL-1 α at 500 pg/ml and rabbit polyclonal anti-human IL-1 α and β antibodies (1 mg/ml, Genzyme, Boston, MA, USA) at 10 µg/ml (n=6), recombinant human TGF-β 2 at 10 ng/ml and rabbit polyclonal anti-human TGF-β 1 and TGF-β 2 antiserum (1 mg/ml, King Brewing Co Ltd, Hyogo, Japan) at 10 µl/ml (n=7), or recombinant human b-FGF at 10 ng/ml and rabbit polyclonal anti-human b-FGF antibody (1 mg/ml, Collaborative Research Inc, Bedford, MA, USA) at 10 µg/ml (n=5).

In the control plates for IL-1 α and b-FGF, an aliquot of culture medium was replaced with 0.5 ml of culture medium containing rabbit IgG (1 mg/ml, Sigma, St Louis, MO, USA) at the concentration of 10 µg/ml (n=5). In the control plates for TGF-β, an aliquot of culture medium was replaced with 0.5 ml

of culture medium containing rabbit serum (NHT, Tokyo) at the concentration of 10 µl/ml (n=5), or 0.5 ml of culture medium containing rabbit polyclonal anti-human TGF-β 1 and TGF-β 2 antiserum at 10 µl/ml (n=5).

SAMPLE COLLECTION

Twenty four hours after treatment in each culture, the incubation medium was withdrawn and frozen immediately at -70°C and kept at this temperature until the measurement of PGE₂. Thus, the amount of PGE₂ which had accumulated in the incubation medium during the 24 h period after treatment was measured. At the end of culture, the number of viable cells was determined according to the previously reported procedure⁵ (as described below), and the PGE₂ concentration was expressed as picograms of PGE₂ per 10⁴ viable cells.

PGE₂ EXTRACTION AND MEASUREMENT

The PGE₂ concentration in the culture medium was measured with a radioimmunoassay (RIA) kit containing ¹²⁵I labelled PGE₂ (New England Nuclear Corp, Boston, MA, USA) according to the assay procedure described in the kit manual, with modifications as reported elsewhere.^{9,10} Briefly, 0.5 ml of culture medium at 4°C was acidified with 0.05 M citrate to pH 3.5. C-18 extraction cartridge columns (each containing 200 mg of Bond Elut, Analytichem International Inc, Harbor City, CA, USA) were first treated with 2 ml of methanol followed by 4 ml of 0.05 M citrate. The acidified medium was then passed through the cartridge and eluted with 2 ml of distilled water, 2 ml of 10% methanol, 2 ml of cyclohexane, and 2 ml of ethylacetate, in that

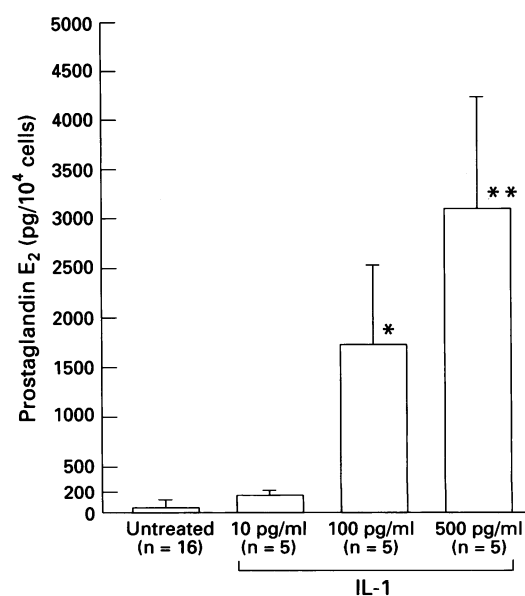


Figure 1 Effect of IL-1 α on the PGE₂ concentration in culture medium containing LECs. The mean PGE₂ concentrations 24 hours after the addition of IL-1 in concentrations of 100 pg/ml and 500 pg/ml were significantly increased. * and ** indicate significant difference at $p < 0.05$ and $p < 0.01$, respectively (multiple comparisons according to Scheffe's method).

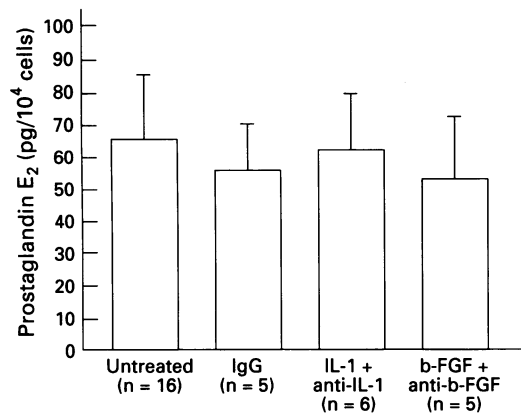


Figure 2 Effect of neutralising anti-IL-1 and anti-b-FGF antibodies on the PGE₂ concentration in culture medium. No significant difference was observed between the PGE₂ concentrations in the experimental and control cultures.

order. The last solvent was evaporated at 37°C under a stream of nitrogen. The minimum detectable amount of PGE₂ by this method was 0.25 pg per tube.

COUNT OF VIABLE CELLS

Without disturbing the cells, the incubation medium was removed from each well, followed by a wash with 0.5 ml of calcium-free and magnesium-free PBS. The cells in each well were then incubated with 0.4 ml of a mixture of 0.25% trypsin and 0.02% ethylene diaminetetra-acetic acid (EDTA) 2 Na for 10 minutes at 37°C. After repeated pipetting to remove the cells from the capsule and the plastic wall of the well, the entire cell suspension was transferred into a small glass test tube, taking care to leave as few cells as possible in the well. The cells were stained by adding 0.1 ml of a mixture of 0.05% crystal violet and 2.1% citric acid, followed by one drop of formalin. Only viable cells could be stained by this procedure. The number of viable cells in the cell suspension from each well was determined, in triplicate, in a Fuchs-Rosenthal cell chamber for counting erythrocytes, and the mean value was used to express the total number of viable cells in each sample.

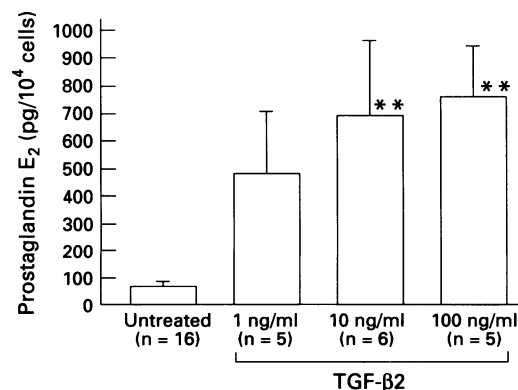


Figure 3 Effect of TGF-β₂ on the PGE₂ concentration in culture medium containing LECs. The mean PGE₂ concentrations after the addition of TGF-β₂ in concentrations of 10 ng/ml and 100 ng/ml were significantly increased.

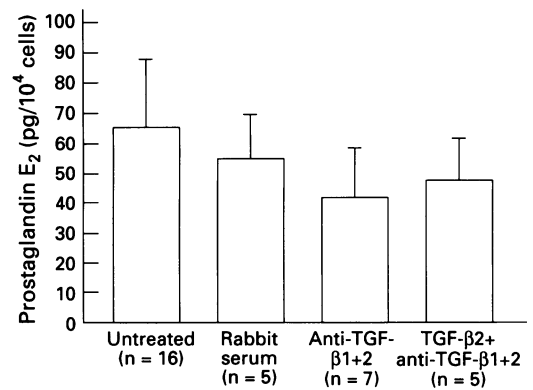


Figure 4 Effect of neutralising anti-TGF-β antiserum on the PGE₂ concentration in culture medium containing LECs. There is no significant difference between the PGE₂ concentration in the experimental and control cultures.

Results

CULTURE OF HUMAN CATARACT LECs

After 3 to 4 weeks, the cells were still proliferating and had become almost confluent in the well. The mean number of viable cells in the untreated cultures and in the treated cultures was 4.25 (0.9) × 10⁴ (mean (SD)) cells (n=16) and 4.07 (1.1) × 10⁴ (n=47), respectively, at the end of culture.

PGE₂ CONCENTRATION IN THE CULTURE MEDIUM

PGE₂ concentration in the culture medium to which IL-1, or IL-1 and its neutralising antibody, was added

The mean PGE₂ concentration in the cultures to which IL-1 α had been added at the concentration of 10 pg/ml was not significant, but at the concentrations of 100 pg/ml (p<0.05) and 500 pg/ml (p<0.01) significantly, increased (multiple comparisons according to Scheffe's method) compared with that in the control cultures (Fig 1). There was no significant difference between the PGE₂ concentration in the cultures to which IL-1 α 500 pg/ml and the neutralising anti-IL-1 α and β antibodies (10 μg/ml) were added and that in the untreated, or rabbit IgG treated controls (Fig 2).

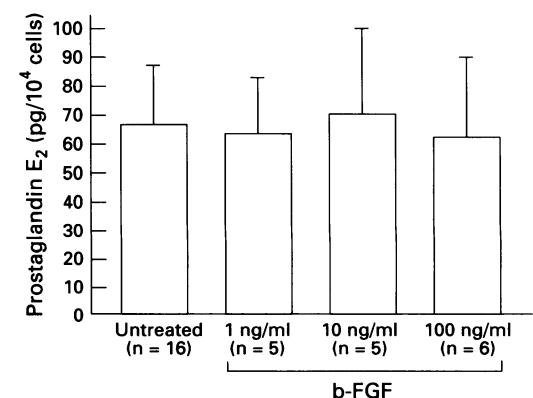


Figure 5 Effect of b-FGF on the PGE₂ concentration in culture medium containing LECs. There is no significant difference between the PGE₂ concentration in the experimental and the control cultures.

PGE₂ concentration in the culture medium to which TGF-β, or TGF-β and its neutralising antibody, was added

The mean PGE₂ concentration in the cultures to which TGF-β 2 had been added at the concentration of 1 ng/ml was not significantly, but at 10 ng/ml ($p < 0.01$) and 100 ng/ml ($p < 0.01$) significantly, increased (Fig 3). There was no significant difference between the PGE₂ concentration in the cultures treated with TGF-β 2 (10 ng/ml) and its neutralising TGF-β antiserum (10 μl/ml) and that in the control cultures treated with rabbit serum, or anti-TGF-β antiserum alone (Fig 4).

PGE₂ concentration in the culture medium to which b-FGF or b-FGF and its neutralising antibody, was added

There was no significant difference between the mean PGE₂ concentration in the medium treated with b-FGF at any concentration and that in the controls (Fig 5). There was no significant difference between the PGE₂ concentration in the cultures to which b-FGF (10 ng/ml) and its neutralising anti-b-FGF antibody (10 μg/ml) were added and that in the untreated, or rabbit IgG treated controls (Fig 2). That the b-FGF preparation used was biologically active was demonstrated by our other experimental studies, in which the same preparation significantly increased the uptake of ³H-thymidine or ³H-proline by LECs of human cataracts in culture (manuscript submitted).

Discussion

The PGE₂ concentration was significantly increased by 24 hours after the addition of human recombinant IL-1 α or TGF-β, and these effects were significantly suppressed by the addition of neutralising anti-human IL-1 or anti-human TGF-β antibodies. The results demonstrate that IL-1 α as well as TGF-β 2 increased PGE₂ synthesis in the primary culture of human cataract LECs. The induction of PGE₂ synthesis by IL-1 in various cells or cell lines has been widely reported. Seckinger *et al*¹¹ reported that PGE₂ production was increased by IL-1 in cultured neonatal mouse calvariae and inhibited by recombinant human IL-1 receptor antagonist. Leizer and Hamilton¹² found that PGE₂ synthesis was increased by IL-1, but that goat anti-IL-1 antiserum significantly inhibited PGE₂ synthesis in the supernatants of human synovial cell cultures. A number of very recent studies¹³⁻¹⁶ have indicated that IL-1 induces PGE₂ synthesis in various kinds of cells.

The mechanism of the increase in PGE₂ concentration by IL-1 or TGF-β may be accounted for by the observations presented in several reports. IL-1 is known to activate *in vitro* phospholipase A₂,¹⁷ cyclo-oxygenase,¹⁸ and lipoxygenase¹⁹ in the arachidonic acid cascade. Some reports indicate that TGF-β increases PGE₂ synthesis by activating cyclo-oxygenase and phospholipase A₂ in lung

fibroblasts and endothelial cells in culture,²⁰ and by activating phospholipase A₂ in rat mesangial cells.²¹ Our results are consistent with these reports. On the other hand, Bry *et al*²² reported that TGF-β inhibits the cytokine (IL-1 and tumour necrosis factor) induced PGE₂ production by cultured amnion cells. However, Kniss *et al*²³ reported that TGF-β increased the cytokine (epidermal growth factor) induced PGE₂ synthesis in mesangial cells. These apparently contradictory findings may be the result of the different cell types or the presence of other modulating cytokines. It would therefore be of interest to study the effect of anti-IL-1 antibodies on TGF-β 2 induced PGE₂ or anti-TGF-β 2 antibodies on IL-1 induced PGE₂ with our culture model of LECs. This might clarify the cytokine context.

We observed no apparent effect of b-FGF on PGE₂ synthesis by human cataract LECs, although there are some reports,²⁴⁻²⁶ that b-FGF increases PGE₂ synthesis. Goddard *et al*²⁷ observed a significant increase of PGE₂ synthesis by b-FGF, which they suggested to activate phospholipase A₂. This discrepancy appears again to be the result of the different cell types and the cytokine context. However, we should take into consideration that in the present study, the investigation was performed at only one time point, which might be the reason that there was no effect of b-FGF observed.

Recent reports have described the detection of IL-1²⁸ in the aqueous humour of rabbit eyes and of IL-6²⁹ in that of human eyes after intraocular lens implantation. IL-6 was observed to cause acute inflammation subsequent to intravitreal injection²⁹ and is known to be induced by IL-1³⁰⁻³¹ in inflammatory host response. TGF-β³²⁻³⁴ as well as b-FGF³⁵ have been detected in the aqueous humour obtained from patients undergoing cataract surgery and in the aqueous humour of the rabbit after IOL implantation.³⁶ Basic FGF was identified immunohistochemically in the proliferated LECs around the lens capsule after cataract surgery.³⁷ Prostaglandin E₂ plays a major role in postoperative inflammation after IOL implantation.³⁸ Lens epithelial cells can be a source of these cytokines and PGE₂ that are present in the aqueous humour *in vivo*. Lens epithelial cells disrupt the blood-aqueous barrier, when they come in contact with the IOL and proliferate after IOL implantation.¹⁻⁴ Our results suggest that endogenous IL-1 and TGF-β produced by LECs act in an autocrine or paracrine manner on PGE₂ synthesis by LECs, which disrupts the blood-aqueous barrier. However, the present study indicates that TGF-β 2 did not significantly alter PGE₂ synthesis at the concentrations present in the normal eye.

In conclusion, IL-1 and TGF-β significantly increased, but b-FGF did not affect PGE₂ synthesis by LECs in culture. The present study clarifies an aspect of our hypothesis regarding postoperative inflammation caused by residual LECs.

The authors have no proprietary interest in the methods and products mentioned in this paper.

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