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Synthesis of interleukin-1 and prostaglandin E_2 by lens epithelial cells of human cataracts*

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Abstract
To test our hypothesis that pseudophakic inflammation, including the fibrin reaction, may be caused by cytokines, prostaglandins (PG), or both, synthesised by residual lens epithelial cells (LECs), we measured interleukin-1α (IL-1α) and PGE_2 in the incubation medium of cultures of human LECs obtained by capsulotomy during cataract surgery. After 1 week radioimmunoassay showed that there were 1·46 (0·62) ng of PGE_2/10^6 cells (mean (SD) six cultures), and after 4 weeks, there were 5·50 (2·20) ng of PGE_2/10^6 cells (seven cultures). During culture the cells proliferated and underwent fibroblast-like cell changes on exposure to the plastic of the wells. In the medium of control plates to which sodium diclofenac had been added PGE_2 was not detected. Some IL-1α was found in four of 10 samples, each of which contained media from 12 cultures; 207 pg/10^6 cells in one of the two pools of 2-week cultures, 120 pg/10^6 cells in one pool and 139 pg/10^6 cells in another of the three pools of 3-week cultures, and 111 pg/10^6 cells in the one pool of 4-week cultures. PGE_2 and IL-1α may therefore be produced in vivo by residual LECs after cataract surgery, and may be involved in postoperative inflammation, including the fibrin reaction.

In our previous clinical study[1] we showed that residual lens epithelial cell (LECs) participated in postoperative inflammation including fibrin reaction[2-5] after intraocular lens implantation.

Aqueous flare intensity (measured by a laser flare cell meter[6]) decreasing from an initial peak owing mainly to surgical trauma, increased again to form a second flare peak, when residual LECs came into contact with the posterior chamber lens and began to undergo fibrous proliferation at 6 to 14 days after surgery. The spike was evidence that the blood-aqueous barrier had been disrupted again. In the eyes, in which residual LECs had been removed by ultrasound aspiration, neither such a flare spike nor fibrous proliferation of residual LECs was noted.

We postulated that residual LECs synthesise prostaglandin E_2 (PGE_2) and cytokines such as interleukin-1 and transforming growth factor, during their fibrous proliferation, and these mediators are responsible for the renewed breakdown of the blood-aqueous barrier.

To test this hypothesis we measured PGE_2 and interleukin-1α (IL-1α) in the incubation media of cultured human LECs, and the results are reported here.

Materials and methods

TISSUE CULTURE OF HUMAN LECS

Circular pieces of the anterior capsule with LECs attached obtained by capsulotomy during cataract surgery were cultured directly without the cells being dispersed. To prevent contamination of the anterior capsules with blood cells the corneoscleral incision was thoroughly cauterised with more than usual care, and after circular capsulorhexis the piece of capsule was touched with an irrigation/aspiration tip and withdrawn from the eye by aspiration. While being held with fine forceps the piece of capsule was thoroughly washed with irrigating solution. Then each piece of anterior capsule was placed immediately into a well of a 48-multiwell plate containing 0·5 ml of Eagle's minimum essential medium containing 10% fetal calf serum, penicillin G at 100 U/ml, and streptomycin sulphate at 100 mg/l. The pieces were observed carefully under an inverted phase-contrast microscope to check for contamination with blood cells, and cultured in 100% humidity at 37°C with a 5% CO_2 atmosphere. The medium was changed every 7 days.

Cell growth in each culture was observed daily under the phase-contrast microscope.

EXPLANTS AND SAMPLE COLLECTION

For the PGE_2 experiment 24 explants from patients with senile immature cataracts were divided into two groups. Cells from 13 explants not treated with sodium diclofenac were cultured as described above; six specimens for 1 week and seven for 4 weeks. Cells from 11 other explants were cultured in the same way except that 10 mg/l sodium diclofenac, a non-steroidal anti-inflammatory drug, was added to each culture. If the medium was changed, sodium diclofenac was added again at the same concentration. Six of the treated specimens were cultured for 1 week and five for 4 weeks. Samples of the culture medium were collected as described below.

For the IL-1α experiment 120 specimens were cultured for 1 to 6 weeks and samples of media were collected. Culture was not continued after the collection. Each sample consisted of media pooled from 12 cultures. Two samples were collected after 1 week of culture, two samples after 2 weeks, three samples after 3 weeks, and one sample after 4, 5, and 6 weeks. At these times the culture medium was sampled with a small pipette and frozen immediately at −20°C. In

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both experiments the cells remaining in each well were counted as described below.

CELL COUNTS

Each well was washed with 0·5 ml of phosphate-buffered saline free from calcium and magnesium, and the cells were then incubated again in the same wells in 0·4 ml of a mixture of 0·25% trypsin and 0·02% EDTA 2Na for 10 min at 37°C. After repeated pipetting to remove cells from the capsule and the plastic of the well, the entire cell suspension was transferred into a small glass test tube, with care to leave as few cells as possible in the well. The cells were stained by the addition of 0·1 ml of a mixture of 0·05% crystal violet and 2·1% citric acid, and then the addition of one drop of formalin. Only viable cells were stained. The viable cells in three samples of medium were counted in a Fuchs-Rosenthal's cell chamber for the counting of erythrocytes.

MEASUREMENT OF PGE_2 AND IL-1α IN THE INCUBATION MEDIUM

PGE_2

The PGE_2 concentration in the culture medium was measured with an RIA kit containing ^{125}I-labelled PGE_2 (New England Nuclear Corp, Boston, MA, USA). We used the assay procedure in the kit manual modified as reported elsewhere.[7 8] In brief, 0·5 ml of culture medium at

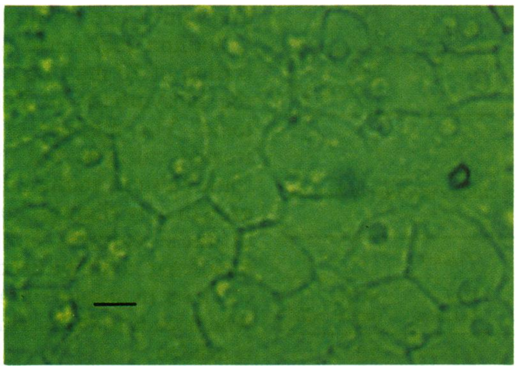

Figure 1 Uncultured human lens epithelial cells observed under an inverted phase-contrast microscope. The cells are mostly hexagonal, and the nuclei and cell boundaries are distinct. (Bar, 12·5 μm.)

4°C was acidified with 0·05 M citrate at pH 3·5. C-18 extraction cartridge columns ((Bond-Elut) 200 mg, Analytichem International, Inc, Harbor City, CA, USA) were first treated with 2 ml of methanol and 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 ethyl acetate, in that order. The last solvent was evaporated at 37°C under a stream of nitrogen.

The minimum detectable amount of PGE_2 by this method was 0·25 pg/tube. Results are given an nanograms of PGE_2 per 10^6 cells.

IL-1α

The culture medium of each sample was concentrated 10 times in a Centricon-10 apparatus (WR Grace and Co, Beverly, MA, USA), so that substances with molecular weights of 10 000 or less were removed. The filtrate was centrifuged at 3000 rpm for 6 hours. The IL-1α concentration in the supernatant was assayed with an IL-1α ELISA kit (Otsuka Pharmaceutical Co, Tokushima, Japan). The minimum detectable amount was 10 ng/l. Results are given as picograms of IL-1 per 10^6 cells.

Results

HUMAN LEC CULTURE

In the first preparations a few blood cells were found in some cultures, which were discarded. The red blood cells could be easily differentiated from LECs, which are polygonal and were mostly attached to the lens capsule. After we took more care with preventing contamination it was no longer detected.

The anterior capsule usually curled upward with the side to which the LECs were attached facing downward, so that the LECs in the centre of the capsule came into direct contact with the well. Where the capsule touched the well, LECs began to proliferate within 2 or 3 days. All proliferating cells lost their initial polygonal shape (Fig 1) and underwent pleomorphic changes, becoming slightly elongated and fibroblastic (Fig 2). As the attached cells underwent these changes and while the areas of attachment were expanding, the anterior capsule became

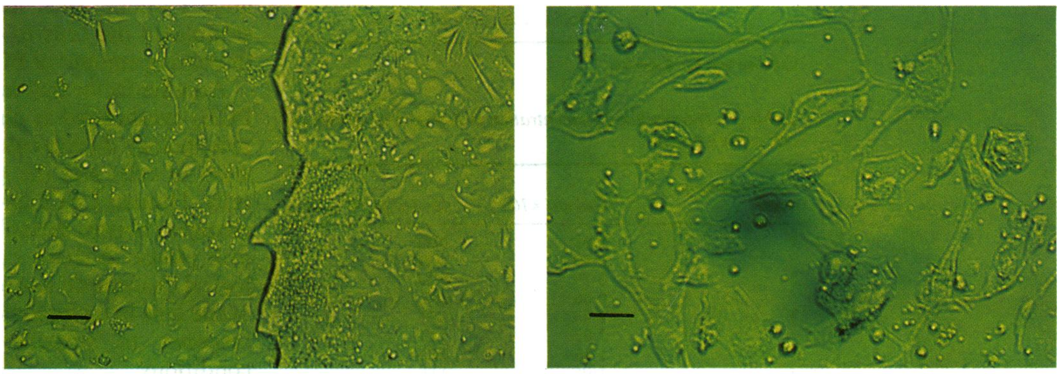

Figure 2 Human lens epithelial cells cultured for 7 days. Left: Lens epithelial cells, which are touching the dish, have started to show pleomorphic changes, and are proliferating in the direction opposite from the capsule on the right. (Bar, 125 μm.) Right: Cells in the area beyond the capsule are elongated and fibroblastic in appearance. (Bar, 50 μm.) The PGE_2 concentration was 2·34 ng/10^6 cells.

fixed to the well and the curled edge gradually flattened. With the pleomorphic changes, the cell boundaries and nuclei became indistinct under the phase-contrast microscope (Fig 2).

From about 1 week after culture began untreated cultured specimens showed cells proliferating to confluence on the capsule, and growth beyond the capsular margin (Fig 2). After 4 weeks the cells were still proliferating and had become almost confluent. In some cultures LEC proliferation was confined to the capsule. Four of the six specimens cultured for 1 week and treated with sodium diclofenac for the PGE_2 measurements were restricted to moderate cell growth compared with the untreated specimens. Of specimens cultured for 4 weeks, treated cultures had fewer cells than untreated cultures (Tables 1A and 1B).

MEASUREMENT OF PGE_2

PGE_2 was detected in the medium from all the untreated cultures. The mean PGE_2 concentration for untreated specimens cultured for 1 week was significantly higher ($p<0.01$, Student's *t* test) than that for untreated cells cultured for 4 weeks (Table 1). However, PGE_2 was detected in only two media from the six treated specimens cultured for 1 week, and the concentration was low, though the cells had slow to moderate growth.

Table 1A PGE_2 concentration in culture medium of human lens epithelial cells

	Untreated		Treated	
Specimen no	Cell numbers	PGE_2 (ng/10^6 cells)	Cell numbers	PGE_2 (ng/10^6 cells)
1	1.7×10^4	0·68	0.7×10^4	0·0
2	2.5×10^4	0·88	1.5×10^4	0·0
3	2.4×10^4	2·34	0.8×10^4	0·18
4	2.6×10^4	1·33	0.8×10^4	0·24
5	1.9×10^4	1·83	1.4×10^4	0·0
6	2.5×10^4	1·71	0.1×10^4	0·0
		1·46 (SD 0·62)		0·07 (SD 0·11)

Culture was for 1 week.

Table 1B PGE_2 concentration in culture medium of human lens epithelial cells

	Untreated		Treated	
Specimen no	Cell numbers	PGE_2 (ng/10^6 cells)	Cell numbers	PGE_2 (ng/10^6 cells)
1	3.5×10^4	2·4	0.7×10^4	0·0
2	2.2×10^4	4·76	0.2×10^3	0·0
3	2.4×10^4	4·27	0.3×10^3	0·0
4	3.5×10^4	7·95	0.2×10^3	0·0
5	3.6×10^4	4·72	1.5×10^4	0·0
6	3.5×10^4	8·21		
7	3.7×10^4	6·59		
		5·50 (SD 2·20; $p<0.01$)		0·0

Culture was for 4 weeks.

Table 2 Interleukin-1 concentration in human lens epithelial cell culture media

Culture duration (week)	Cell no ($\times10^5$)	IL-1α (pg/10^6 cells)
1	0·65	ND
	0·40	ND
2	0·79	ND
	0·86	207
3	0·83	120
	1·03	139
	1·16	ND
4	1·05	111
5	0·91	ND
6	0·85	ND

ND=not detected.

MEASUREMENT OF IL-1α

IL-1α was detected in one of the two samples of specimens cultured for 2 weeks, in two of three samples cultured for 3 weeks, and in the one sample cultured for 4 weeks (Table 2). In the control media without LECs, IL-1α was not detected.

Discussion

PGE_2 is synthesised by various ocular tissues[9-13] and is important in intraocular inflammation because it disrupts the blood aqueous barrier. There are only a few reports on the possible contribution of the crystalline lens to PG biosynthesis,[14-19] and the lenses of some species have been found not to produce PGs.[14 15]

Taylor *et al*[16] did not detect PGs in the medium of cultured calf LECs by RIA. However, Belisle *et al*[17] presented evidence suggesting that rat LECs synthesise PGs. Keeting *et al*[18] reported on PGE_2 synthesis by homogenised rat lenses. Fleisher and McGahan[19 20] reported on PGE_2 synthesis by lenses from experimentally inflamed rabbit eyes. There are no published reports of PG synthesis by human LECs.

Here we found that PGE_2 was synthesised by epithelial cells of human crystalline lens from patients with cataracts. PGE_2 was detected in every untreated medium.

Sodium diclofenac was used at the concentration of 10 mg/l because this dose suppresses the biosynthesis of PGE_2 without being very toxic. The number of cells was small in the treated cultures, but viable cells remained. In some cultures sodium diclofenac suppressed cell growth only slightly, but PGE_2 was not detected in these cultures either. Therefore 10 mg/l diclofenac could be used in the controls.

To set in motion the arachidonic acid cascade, which leads to PGE_2 synthesis, some stimulation is needed.[21]

Tissue injury during surgery and cell culture preparation could affect the PGE_2 concentration in the culture medium. However, as we found increased PGE_2 concentrations in older cultures after 4 weeks, such injury is unlikely to have stimulated the cells to continue to produce the increasing amount of PGE_2 detected.

Another factor that might affect the amount of PGE_2 synthesised is changes in tissue and cell morphology during culture; that is, the proliferation of cells and the growth of fibroblast-like cells. Human LECs undergo fibroblast-like cell changes (fibrous metaplasia) on exposure to the dish plastic in tissue culture.[22-24] Such morphological changes do not resemble the normal state of the lens in an intact lens capsule, but reflect a response to stimulation or to pathological conditions.

Our results therefore suggest that exposure of human LECs to the plastic of the well stimulated PGE_2 synthesis in tissue culture. Further, in untreated cultures, the PGE_2 concentration at 4 weeks was significantly greater than that at 1 week, so the rate of synthesis of PGE_2 by LECs was greater at 4 weeks than at 1 week. We postulate that it increases as LEC proliferation, with fibroblast-like cell change, advances.

The cytokine IL-1 is one of the peptide mediators involved in both the up and down regulation of immunological, inflammatory, and reparative host responses to injury; the cytokines act at concentrations of 10^{-10} to 10^{-15} mol/l.[25 26] Because these small quantities impede the purification, and the minimum detectable amount for IL-1 was 10 ng/l, we pooled the incubation media of 12 cultures and concentrated the pool obtained 10 times to make one sample.

IL-1 was detected in four untreated samples after 2, 3, or 4 weeks. That IL-1α was not detected in the six other samples does not prove that IL-1α was not synthesised in those cultures. It may not have reached a detectable level in the earlier cultures.

IL-1 is produced by monocytes, macrophages, lymphocytes, epithelial cells, endothelial cells, fibroblasts, and synovial cells.[26] There have been no reports of IL-1 synthesis by LECs of any species, including humans.

Most normal cells that can produce IL-1 will do so only when stimulated by one of a variety of agents.[26] Here, as in PGE_2 synthesis, contact between the LECs and the well plastic may have been a stimulant to IL-1 synthesis.

IL-1α increases PGE_2 synthesis[25] by activating phospholipase,[27] lipoxygenase,[28] and cyclogenase[29] in the arachidonic acid cascade. We therefore think that IL-1 was first produced on exposure to the well plastic, and it stimulated the LECs to synthesise PGE_2.

The tissue culture that we used, with human LECs attached to the anterior capsule cultured directly without cell dispersion, resembles the in vivo condition after cataract surgery. After cataract surgery residual LECs proliferate, showing fibrous metaplasia in the defective, avascular lens capsular bag containing an intraocular plastic lens. This condition is analogous to the tissue culture model used, in which LECs attached to the torn anterior capsule proliferate and show fibroblast-like cell change on exposure to the plastic of the wells.

Moreover, our report[1] that the blood-aqueous barrier was disrupted again at 6 to 14 days postoperatively while residual LECs underwent fibrous proliferation at the defective capsular margin after intraocular lens implantation is consistent with the present results.

That PGE_2 and IL-1α are detected in the culture media suggests that, in vivo also, residual LECs may synthesise these compounds.

In conclusion, IL-1α and PGE_2 were detected in culture media of human LECs and could be produced also in vivo by residual LECs after cataract surgery. They may have an important role in the pathogenesis of postoperative inflammation, in aphakic and pseudophakic eyes.

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