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Lens Epithelial Cells in Postoperative Aqueous Humor

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Abstract: The aqueous humor during or after cataract surgery often contains red blood cells (RBCs) from blood; inflammatory cells from white blood cells (WBCs); fibrin, pigment cells, and fibroblasts from either the iris or the ciliary body; lens epithelial cells (LECs); and corneal endothelial cells. Since LECs may be related to postoperative complications, including a fibrin reaction and a secondary cataract, confirmation of their presence postoperatively not only in the capsular bag, but also in the aqueous humor, is necessary for identifying these complications. Using aqueous humor obtained from pig eyes, I attempted to identify LECs immunohistochemically in the aqueous humor, following phacoemulsification (PEA) and aspiration of the cortex, and found the presence of cells that react to keratin antibody, the marker for epithelial cells. Although these cells could be lens, conjunctival, or corneal epithelial cells, they were found following PEA and aspiration of the cortex, and therefore are believed to be LECs. **Jpn J Ophthalmol 1997;41:55–58** © 1997 Japanese Ophthalmological Society

Key Words: Aqueous humor, cataract surgery, immunohistochemical method, lens epithelial cells, pig eyes.

Introduction

Because current cataract surgical procedures are almost always followed by implantation of an intraocular lens (IOL), studies are frequently done to evaluate the biocompatibility of various IOL materials. However, even the most biocompatible material will always be regarded as a foreign body and provoke a cell reaction on its surface. These cells can be examined by specular microscopy[1], implant cytology (a method of staining the extracted IOL with hematoxylin eosin),[2–5] scanning electron microscopy,[6,7] and transmission electron microscopy.[8,9] However, deposits found in the anterior segment following IOL implantation may be red blood cells (RBCs) originating in the blood, inflammatory cells originating in white blood cells (WBCs), fibrin, pigment cells and fibroblasts from either the iris or ciliary body, lens epithelial cells (LECs), or corneal endothelial cells. Of these, it appears that LECs, possibly detaching from the anterior capsule and floating in the aqueous during or following PEA, aspiration of the cortex, or IOL implantation, may provoke various postoperative complications such as secondary cataract,[10–12] and synthesize cytokines, leading to postoperative inflammation.[13,14] Until recently, LECs remaining after surgery have been regarded as cells adhering to the anterior lens capsule; there are some studies reporting the correlation of these cells to secondary cataract[10–12] and postoperative inflammation.[13,14] Other studies evaluating the possibility of LECs in the aqueous humor following surgery have been neglected, so there are no reports available to date confirming their presence in the aqueous humor.

If LECs are present in the aqueous humor, they are likely to elongate and proliferate on the implanted IOL as well as on the posterior lens capsule.[15] These cells, and the chemical mediator they induce, may be implicated in a number of postoperative complications. For this reason, I studied the presence of LECs immunohistochemically, using the aqueous humor obtained by PEA and aspiration of the cortex of pig eyes.

Materials and Methods

Eyes were enucleated from pigs obtained from a local abattoir. Lenses were extracted from 20 eyes within 2 hours of enucleation, during which time

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they were refrigerated at 9°C. A fornix-based flap was created to expose the sclera, and each eye was washed with a saline solution. Two millimeters posterior from the surgical limbus, the anterior chamber was entered via a straight scleral incision, 3 mm wide, made with a 3 mm straight slit blade (Beaver, Franklin Lakes, NJ, USA). A viscoelastic material was then injected into the anterior chamber and, using a 23-gauge needle, continuous curvelinear capsulorhexis was done. Phacoemulsion (PEA), using 10,000 Master HydroSonics (Alcon Surgical, Fort Worth, TX, USA) was followed by aspiration of the cortex. Ringer's solution (Otsuka Pharmaceutical, Tokyo, Japan) was used as the irrigating solution.

A syringe with a 23-gauge needle was used to aspirate 0.2 mL of aqueous from each eye; 1 mL of aqueous was immediately centrifuged for 10 minutes at 15,000 cycles/minute using a high-speed micro-refrigerated centrifuge (MTX-150, Tomy, Tokyo). Live cells were identified by trypan blue staining: 3 mL of aqueous was fixed with 5% formaldehyde and centrifuged for 10 minutes at 15,000 cycles/minute as above; 1.0 mL of Ca^{2+}, Mg^{2+}-free phosphate buffered solution (PBS) was added to the cells; 7 μL of sample was removed for cell counting with a hemocytometer. The remaining sample was again centrifuged for 10 minutes at 15,000 cycles/minute; gathered cells were mixed with 1.0 mL of 0.1% bovine serum albumin, adjusted with PBS, lightly pipetted, blocked for 30 minutes at room temperature, then centrifuged again for 10 minutes at 15,000 cycles/minute. Cells were reacted for 1 hour with rabbit polyclonal antikeratin (Dako, Carpinteria, CA, USA) diluted to 1/200 with PBS, and used as the primary antibody. This antibody had been verified as reacting with the LECs of pig eyes (Figure 1) in another set of experiments.

Biotin-marked anti-mouse IgG, rabbit IgG goat serum (Nichirei, Tokyo, Japan) was added to the sample; after 10 minutes of reaction, the sample was washed with PBS and reacted for 5 minutes with peroxidase-marked streptoavidin (Nichirei, Tokyo, Japan); the cells were stained with 3-amino-9-ethylcarbazole (AEC staining, Nichirei, Tokyo, Japan) and examined under a light microscope on a glass slide. For the negative control, the gathered cells were treated as above, with normal rabbit serum used as the primary antibody.

From 20 pig eyes obtained in the same manner and kept at 9°C for 2 hours following enucleation, a total of 4 mL (0.2 mL from each eye) was extracted with a 23-gauge needle, and care was taken not to damage the cornea, iris, or lens. The aqueous humor was centrifuged for 10 minutes at 15,000 cycles/minute and the supernatant discarded. After adding 50 μL of PBS and pipetting, 10 μL of sample was placed on a glass slide and examined under a light microscope for any identifiable cellular components possibly released by postmortem changes. This step was taken to determine if any cells existed in the aqueous humor of eyes not being subjected to a lens extraction procedure.

Results

Before fixing with 5% formaldehyde, the cells obtained after centrifugation were stained with trypan blue, none turned blue, indicating that they were all live cells (Figure 2). After fixation with 5% formaldehyde, the number of cells obtained by centrifugation was 2×10^5 cells/mL. Immunologic staining ver-

Figure 1. Pig LECs stained with immunohistochemical method (× 300).

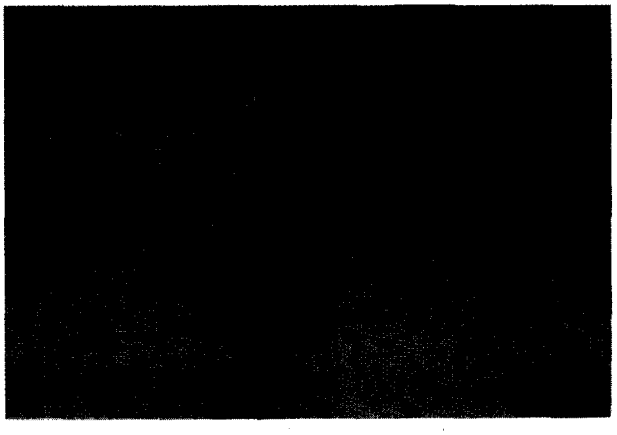

Figure 2. Arrows indicate cells not stained using trypan blue (alive cells) (× 150).

Figure 3. Stained cells were observed and presence of LECs was confirmed in aqueous humor after surgery (× 300).

ified that some cells that reacted with polyclonal antikeratin antibody were epithelial cells (Figure 3), but it was not possible to determine the percentage of epithelial cells in 2×10^5 cells/mL. In the negative control, none of the cells stained red (Figure 4). In the same manner, 4 mL of aqueous humor obtained from pig eyes not subjected to PEA, aspiration of the cortex, or lens extraction contained 1.2×10^4 cells/mL. These cells appeared to be melanin cells, possibly originating from trauma to the iris during aqueous humor aspiration or from postmortem changes causing iris melanin cells to be released into the aqueous humor (Figure 5). Further studies are necessary to evaluate the origin of melanin cells.

Discussion

Cataract surgical procedures, equipment and prosthetics are continually being refined; this rapid development has already produced phacoemulsification, small incision procedures, foldable IOLs, and

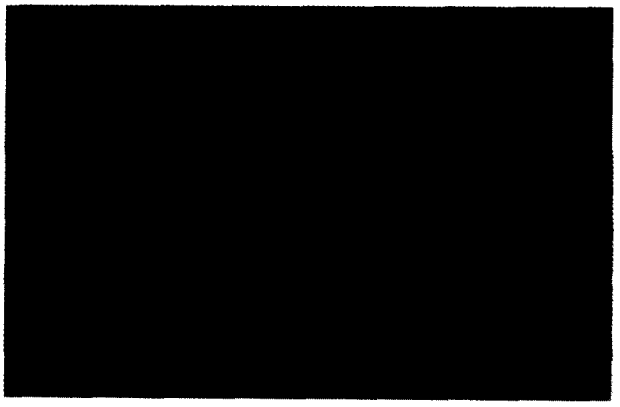

Figure 4. In the negative control, stained cells were not observed (× 300).

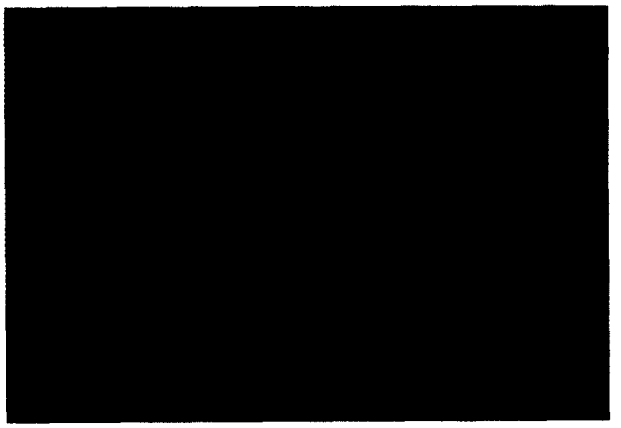

Figure 5. Only melanin cells were observed in aqueous humor (× 100).

sutureless methods, all of which enable more institutions and clinics to do procedures on an outpatient basis.

Cataract extraction and IOL implantation procedures are not without complications, however, and one of the major postoperative complications is secondary cataract, seen in almost all cases. Although residual LECs have now been identified as the cause of this complication,[10–12] the mechanism remains to be defined. Currently, the only way to treat the complication is with YAG laser or surgical incision, or by removal of the posterior capsule.

Fibrin reaction, although infrequent, is another postoperative complication that cannot be ignored. It also is induced by chemical mediators synthesized by LECs after IOL implantation procedures.[13,14] Until recently, LECs remaining after surgery have been regarded as cells adhering to the anterior lens capsule. Procedures such as lens extraction, PEA, aspiration of the cortex, and IOL implantation may cause the cells to detach from the anterior lens capsule and be released into the aqueous humor. This cell activity must be considered in evaluating postoperative complications.

PEA and aspiration of the cortex were used to determine, immunohistochemically, if the aqueous humor of enucleated pig cadaver eyes contained any LECs and if polyclonal antikeratin, which reacts with epithelial cells, would react with the LECs of pig eyes. Cells reacting to the antibody were found, having turned red with AEC stain. Only melanin cells were detected in the aqueous humor of pig eyes that had no surgery, confirming that postmortem changes were not responsible for LECs in the aqueous humor.

However, stained cells or the epithelial cells in the aqueous humor might be cornea or conjunctival epi-

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thelial cells, but neither is likely with the surgical procedures used in this study; origin in the lens is the most reasonable conclusion. The effect of postmortem changes in the pig eye appears to be minimal since none of the cells in the aqueous humor were stained with trypan blue immediately after lens extraction.

This study involved lens removal by PEA and aspiration of the cortex; the results confirmed the presence of live cells in the aqueous humor immediately after these surgical procedures. Since these cells have high potential for adhering, elongating, and proliferating onto the IOL and the posterior lens capsule, the possibility of similar LEC activity by those cells remaining on the anterior lens capsule and in the aqueous humor should be considered in evaluating their involvement in the development of postoperative complications.

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