tested. This is consistent with results reported by Kaye et al., who found no changes in rabbit endothelium exposed to pressures of 60 to 80 mm Hg.

This study demonstrates that rabbit corneal endothelium functions normally in the presence of elevated hydrostatic pressure. Regenerated rabbit endothelium functions similarly to normal endothelium in the presence of elevated hydrostatic pressure.

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Key words: cornea, corneal endothelium, intraocular pressure, rabbit

REFERENCES


Epithelialization of the corneal endothelium in posterior polymorphous dystrophy. Merlyn M. Rodrigues, Tung-Tien Sun, Jay Krammer, and David Newsome.

The unusual cell type present on the posterior corneal surface of posterior polymorphous dystrophy patients has been characterized. In addition to microvilli and desmosomes, these cells contain abundant 10 nm filaments which by immunofluorescent staining were shown to consist of keratin proteins, a marker for epithelial cells.

Posterior polymorphous dystrophy (PPMD) of the cornea is a disorder of the corneal endothelium usually associated with autosomal dominant inheritance. Clinically this entity is characterized by bilateral involvement of the posterior cornea. Lesions can range from tiny vesicular areas, thickening, and bands to advanced posterior corneal changes with secondary stromal and epithelial edema, necessitating corneal transplantation. Congenital posterior corneal vesicles have been observed in offspring of families with this condition. Histologically PPMD is associated with irregular multilaminar Descemet's membrane which displays normal anterior banding as well as abnormal cells present in the endothelial layer.

Recently it has been shown that keratin proteins are present in the form of tonofilaments not only in epidermal cells but also in corneal epithelial and a wide variety of other epithelial cells. Of particular interest is the fact that when frozen sections of cornea were stained with antikeratin antiserum by indirect immunofluorescent staining, it was found that corneal epithelium was the only cell type that stained; no staining could be detected in the stroma or endothelium.

We now report that the unusual epithelial-like cells in the corneal endothelial layer in PPMD can be stained specifically with antikeratin antiserum and thus that they contain keratin filaments, a marker for epithelial cells.

Materials and methods. Antibodies prepared against a group of keratins purified from human stratum corneum were used to identify epithelial cells containing keratins by immunofluorescence. The antiserum against the keratin fraction was characterized by immunoelectrophoresis with sodium dodecyl sulfate gel and contained antibodies against all the major electrophoretic bands of keratins. For immunofluorescent staining, frozen sections (6 μm thick) were prepared. The air-dried tissue sections were hydrated in phosphate-buffered saline (PBS) and covered with 20 μl of antikeratin antiserum previously diluted 1:48 with PBS. The sections were incubated in a humidified chamber at 37°C for 30 min. They were then washed in three changes of PBS for a total of 30 min, overlaid with fluorescein-conjugated goat anti-rabbit IgG (1:16 diluted; Miles Laboratories, Inc.) and incubated at 37°C for 30 min. After they were rinsed again, these speci-
Fig. 1. Scanning electron micrograph of corneal tissue specimen (keratoplasty) in PPMD. Cells resembling "normal" corneal endothelium (N-ENDO) adjacent to large "transformed" endothelial-like cells (T-ENDO). (×1800.)

imens were mounted in Gelvatol11 and viewed in a Leitz Orthoplan microscope with epi-illumination. Photographs were taken with Ektachrome 400 ASA film with Tri-X pan film.

For tissue culture studies, explants of corneal epithelium, stroma, and endothelium from the PPMD patient as well as from normal human were maintained in 60 mm polystyrene dishes with Eagle's minimal essential medium containing 5% fetal calf serum, penicillin (100 U/ml), and streptomycin (50 μg/ml). The dishes were kept at 37° C in 95% air–5% CO2 and 100% humidity. Cultured endothelial and epithelial cells were rinsed in PBS, placed in methanol, chilled to -20° for 5 min, reacted with antikeratin antiserum as described,10 and viewed with epifluorescent illumination.

Scanning electron microscopy was performed on corneal specimens fixed in 3% buffered glutaraldehyde, postfixed, in osmium tetroxide, dehydrated in graded acetone, critical point-dried, and double-coated with carbon and gold palladium. For transmission electron microscopy, tissues were fixed in 2.5% buffered glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated through ascending alcohols, and embedded in epoxy resin.

Results. Scanning electron microscopy of the PPMD corneal specimen disclosed endothelial cells with hexagonal configurations and scant microvillous projections adjacent to large epithelial cells with numerous microvilli (Fig. 1).

Transmission electron microscopy of the PPMD corneal endothelial tissue (Fig. 2) revealed Descemet's membrane lined with epithelial-like cells showing numerous prominent desmosomal attachments, cytoplasmic filaments measuring 8 to 10 nm in diameter, numerous microvillous projections, and scant mitochondria.

Consistent with previous findings,5 when frozen sections of corneas were stained by indirect immunofluorescence with antikeratin antiserum, the epithelium of normal human cornea and the specimen of Fuch's corneal endothelial dystrophy displayed intense fluorescence in all cell layers; no fluorescence was observed in the corneal stroma and endothelium. The epithelium of the PPMD specimen showed fluorescence similar to that of the control cornea. However, in the PPMD corneal endothelial layer, cells stained strongly by antikeratin could be observed (Fig. 3). These cells were present in an unusual patchy pattern (Fig. 3), with occasional nonfluorescent areas corresponding to foci where presumably normal endothelial cells persisted. Immunofluorescent staining of
Fig. 2. Transmission electron micrograph of corneal endothelium in PPMD. Prominent desmosomes (box) are present adjacent to cytoplasmic filaments (arrow) measuring 8 nm in diameter. DM, Descemet's membrane. (×45,600.)

Fig. 3. Corneal tissue (keratoplasty specimen) from a patient with PPMD, stained with antikeratin antibody. Normal immunofluorescence of the epithelium (E) and abnormal patchy immunofluorescence of the endothelium (arrows) is present. The fluorescent areas of the endothelium (arrows) correspond to cells with epithelial transformation and the nonfluorescent patches to residual endothelial cells (×50.) Inset, Marked cytoplasmic fluorescence of "endothelial" cells with epithelial transformation (arrow). (×100.)

Fig. 4. Cell cultures of the PPMD corneal endothelium stained with antikeratin antibody. Transformed endothelial cells display intercellular bridges and immunofluorescence typical of epithelial cells. (×100.) Inset at higher magnification shows large squame-like cell with keratin fibers. (×160.)

cells cultured from the PPMD corneal endothelial tissues with antikeratin antiserum showed numerous large flat cells containing delicate cytoplasmic networks of wavy fibers (Fig. 4). Nonfluorescent cells were also observed and probably represent relatively normal corneal endothelial cells.

Discussion. The corneal endothelium is derived either from neural crest cells or from primitive mesenchyme. Normal human corneal endothelium is composed of a monolayer of hexagonal cells with occasional microvillus projections, abundant mitochondria, and apical gap junctions. In PPMD the corneal endothelium is frequently a double layer of cells with myriad microvillus projections, scant mitochondria, numerous prominent desmosomal junctions, and intermediate filaments measuring 8 to 10 nm in diameter. It has been established that accidental or surgical trauma can introduce normal surface epithelium of conjunctival or corneal origin into the anterior chamber. In PPMD, however, the unusual corneal endothelial changes occur without such trauma.

The derivation of this unusual epithelialization of the corneal endothelium in PPMD is uncertain and is suggestive of a developmental anomaly involving transformation of cells across germinal lines or of a persistence of embryonal cell rests. Such a marked change in the pathway of differentiation would most likely occur during ges-
Number 6 and has been observed at birth. In PPMD, Descemet's membrane displays anterior banding, usually occurring during the fifth month of gestation, but lack of a uniform posterior granular layer suggests intrauterine damage to the corneal endothelium. If the abnormal corneal endothelial cells were displaced ectodermal cells, they would most likely have expressed their dysfunction at the same time as other derivatives of surface ectoderm, which are unaffected in this disease. All these factors are consistent with a most unusual transformation of corneal endothelial cells into keratin-containing epithelial cells. We have demonstrated that this change of cell type, an important feature of PPMD, distinguishes it from normal human corneal endothelium and from other disease of the corneal endothelium such as Fuchs' dystrophy.

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Key words: posterior polymorphous corneal dystrophy, keratin, immunofluorescence, epithelialization

REFERENCES


Concentration-dependent morphologic effects of cytochalasin B in the aqueous outflow system. MURRAY JOHNSTONE, DAWN TANNER, BRUCE CHAU, AND KENNETH Kopecky.

Cytochalasin B at concentrations of 1, 5, 10, 15, 20, or 40 μg/ml was continuously exchange-perfused into the eyes of nine living Macaca mulatta monkeys while intraocular pressure (IOP) was maintained at 25 mm Hg for 30 min. Pressures were then slowly reduced to 4 mm Hg to permit blood to reflux into Schlemm's canal as a tracer, and the eyes were fixed while maintained at 4 mm Hg IOP. Tissues were examined in all eyes by light and transmission electron microscopy. At concentrations of 1, 5, and 10 μg/ml cytochalasin B, the integrity of the endothelial lining of the trabecular wall of Schlemm's canal was maintained. At 15 μg/ml cytochalasin B, 6% of the length of the endothelial lining was disrupted; at 20 μg/ml, 54%; and at 40 μg/ml, 83%. There was a washout of extracellular material at the site of breaks and also a reflux of blood from Schlemm's canal into the trabecular meshwork in the same regions.

Intracameral infusion of cytochalasin B (CB) causes a large increase in gross outflow facility in the cynomolgus monkey. Since ruptures in the endothelial lining of the inner wall of Schlemm's...