Epithelial Characteristics of the Endothelium in Chandler’s Syndrome

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The cells lining the posterior surface of a corneal button obtained from a patient with Chandler’s syndrome were examined by transmission and scanning electron microscopy, and by keratin staining. The ultrastructural appearance with intracytoplasmic 8 nm filaments, desmosomes, microvillous projections, and positive keratin staining suggests that these may be epithelial cells. Clinical specular microscopy of this cornea prior to transplantation revealed characteristic changes that were seen to be related to the density of microvillous projections on the cell membrane. Invest Ophthalmol Vis Sci 24:603–611, 1983

Case Report

A 68-year-old white man was first seen at the Wilmer Institute in November 1975 because of a superficial corneal foreign body of the left eye. He had a past history of spontaneous corneal edema of the left eye in 1970, which resolved during 5 days without treatment, and during which time his intraocular pressure remained 17 mmHg. In 1974 he had a similar corneal edema of the left eye, with a pressure of 12 mmHg. Between these episodes he was followed up regularly and was apparently found to have normal intraocular pressure (IOP).

On examination in 1975, his vision was 20/25 in the right eye and 20/25 in the left. He had a clear right cornea with no endothelial abnormalities noted by slit-lamp examination and a clear anterior chamber. The right lens showed +1 nuclear sclerosis. He was next seen in 1977 with acute onset of pain in his left eye and visual blurring, an IOP of 50 mmHg, and corneal edema diagnosed as a result of acute angle-closure glaucoma. A peripheral iridectomy was performed, and his IOP remained normal after surgery with topical antiglaucoma medications. When re-examined in October 1981, because of decreasing vision in his left eye, the vision was 20/25 in the right eye and light perception with accurate projection in the left eye. The right eye had a clear cornea (Fig. 1) with a grade 3 open angle in all areas and an IOP of 18 mmHg by application. In the left eye, band keratopathy was increased, and it obscured the view of the central corneal endothelium (Fig. 1). Peripheral corneal endothelium by slit-lamp examination had a guttate appearance. The anterior chamber was deep, and a peripheral iridectomy was present at the 1-o’clock position. There was ectropion of the iris pigment layer at the 12-o’clock position, and inferiorly at the 5:30-position in the midperiphery there appeared to be an iris nevus (Fig. 1). Gonioscopy revealed peripheral anterior synechia (PAS) for almost 270°, extending anteriorly in front of Schwalbe’s line. The left pupil dilated fully, and he was noted to have a dense nuclear sclerotic cataract. Ophthalmoscopic examination was not possible because of the media, but ultrasound

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suggested no evidence of gross retinal pathology. Examination was also performed with a small-field specular microscope and with a wide-field specular microscope.

He had a left penetrating keratoplasty and extracapsular cataract extraction performed on the left eye in December 1981. Three quarters of the corneal button, the broad iridectomy specimen, and a conjunctival biopsy specimen were fixed in glutaraldehyde-formaldehyde solution for light microscopy and ultrastructural examination. A rim of the donor corneal tissue and the remaining quarter of the patient’s corneal button were frozen and sectioned for antikeratin antibody staining and monoclonal antikeratin antibody staining (by methods from personal communication with Tung Tien Sun, PhD.

Of the patient’s 23 living relatives, 20 were given a slit-lamp examination of the anterior segment.

Results

Clinical Examinations

Family examination: Of the 20 family members examined there was no history of glaucoma in any, and slit-lamp examination disclosed the corneas, endothelial surfaces, and iris details in all members to be normal. One relative had map-dot-fingerprint corneal changes and cataracts, and in another, some very fine pupillary remnants associated with an anterior polar cataract. Their ages ranged from 5 years to 78 years. One daughter and her two children, who had no history of ocular disease, were not available for examination. No evidence of minor posterior polymorphous endothelial dystrophic changes was seen on the endothelial surface of any of the family members.

Specular microscopy of the patient: Both small-field and wide-field specular microscopy of the patient’s unaffected right eye revealed a normal endothelial surface, with perhaps some increased pleomorphism of endothelial cells (Figs. 2A, B). The entire central 8 to 10 mm of corneal endothelium was scanned with the wide-field specular microscope to confirm the absence of any discrete abnormalities.

In the left eye, only the areas of corneal endothelium above and below and to the sides of the band keratopathy could be viewed, and in all areas that were visible by the wide-field unit there was no evidence of normal endothelium (Figs. 3A, B). Reversal of cell outlines and variations of cell shape were prominent, with a diffuse uniform involvement in all areas observed. Small-field specular microscopy at high magnification disclosed some cell boundary outlines, but mainly a reversal of cell outlines, the center of the cell being dark and the periphery of the cell being light, with some highlighted refractile edges (Fig. 3B).

Histopathology

Light microscopy: Cornea. The corneal button showed slight irregularity of the epithelial surface, and the basement membrane was fibrillated, with a minor degree of fibrous pannus formation between this and Bowman’s layer. The stroma was normal. Descemet’s membrane was very thin, and the endothelial cell layer was mildly attenuated. In the center of the button, 19 nuclei of endothelial cells were counted in the view of a single high-power field (×400).

Iris: Light microscopic examination of 1-micron thick sections in epoxy resin (Epon) disclosed a small fragment of iris with a single layer of cells with an apparent basement membrane on the anterior surface. Occasional pigment granules were present in these cells.

Transmission electron microscopy: Cornea. Descemet’s membrane was thin throughout and measured about 2.5 microns in thickness. The cells lining the posterior surface were mostly in a single layer (Fig. 4), but showed some overlapping in areas, and in occasional areas were arranged in two layers. No distinct basement membrane was present where the cells were apposed to Descemet’s membrane. The internal portion of Descemet’s membrane was, however, composed of a thin fibrillar basement membrane-like material. The endothelial cells had some characteristics of epithelial cells (Fig. 4). These consisted of numerous filaments (keratofibrils) that averaged 8 nm in diameter (Fig. 4). These cells adjacent to Descemet’s membrane contained numerous free ribosomes and glycogen particles. A moderate number of mitochondria and pinocytotic vesicles were present. In some cells the pinocytotic vesicles were

Fig. 1. Right eye of patient, with a normal cornea and iris. Left eye of patient showing central band keratopathy, ektropion of the iris pigment layer at the 12-o’clock position, and one iris nevus at the 5:30-position.
quite numerous. The cells composing the layer facing the anterior chamber contained sparse, but well-developed, microvilli. There were well-developed desmosomal attachments in some areas (Fig. 4). Other attachments were composed of a placoid density of the cellular membranes and a space between the cells. The innermost cells also contained a few keratohyaline granules and had intracytoplasmic features that were similar to the cells next to Descemet's membrane. The cell processes were serpiginous and well approximated.

Iris. The anterior surface of the iris was covered by a monolayer of cells that showed polarity with a moderate number of surface microvilli and well-defined single or multiple layers of basal basement membrane measuring from 0.1 to 0.5 micron in thickness (Fig. 5). The cells had numerous, well-developed desmosomal attachments near the apical borders (Fig. 5).
These cells contained numerous filaments that measured 8 to 9 nm in diameter, numerous free ribosomes and glycogen particles, and rarely mitochondria (Fig. 5). No distinct keratohyaline granules were seen.

**Scanning electron microscopy: Cornea.** The cell layer lining the posterior corneal surface showed cell pleomorphism with no regular hexagonal array (Fig. 6). Most noticeable were the microvillous projections on most cells, with some cells showing a predominance of microvillous projections around their periphery near the intercellular boundaries and others showing this predominance centrally (Fig. 6). The cells with mainly peripheral microvillous projections...
Fig. 4. Posterior surface of the thin Descemet's membrane (arrowheads) is lined by one (upper inset) or two layers (upper photograph) of cells that are joined by desmosomes (circle and lower photograph), contain cytoplasmic filaments (arrows, upper and lower photos) that measured 8 nm in diameter and are arranged in fusiform aggregates, and have microvillous projections (asterisk) (upper, X21,000; lower, X104,000; inset, para-phenylenediamine X480).

appeared to have a nuclear-like mound that was microvillous-free centrally.

Antikeratin antibody staining: Rabbit preimmune serum revealed no staining of either surface of the control cornea. Using rabbit antikeratin antibodies at a dilution of 1 to 50, the control cornea (from the corneal rim of the donor button) was noted to fluoresce strongly on the epithelium with no fluorescence on the endothelial layer. With rabbit preimmune serum, the patient's cornea showed no fluorescent staining of either surface. With the rabbit antikeratin antibody, however, strong fluorescence was noted on both the surface epithelium and the posterior cellular layer (Fig. 7). There were no areas of endothelial cells that did not take up the fluorescent stain strongly.

Monoclonal antibody staining to keratin showed positive fluorescence with antibody AE1, AE3, and AE4.

Discussion

The clinical features in our case included: two documented episodes of unilateral corneal edema with normal IOP and spontaneous resolution of the edema;
evidence of unilateral IOP rise, and abnormal endothelial surface by slit-lamp examination, in 1975; increased IOP requiring iridectomy and further medical glaucoma therapy in 1977; broad PAS formation for 270°, anterior to Schwalbe's line; ectropion of iris pigment layer and iris nevus formation; characteristic features on specular microscopy of the involved eye and normal endothelium in the uninvolved eye; and, lack of corneal and iris abnormalities and glaucoma in 20 out of 23 of the patient's living relatives.

All of these features corroborate the clinical diagnosis of Chandler's syndrome of the left eye. The presence of a surgical iridectomy in 1977 raises the possibility that this patient has an epithelial ingrowth. The existence of corneal disease, including recurrent corneal edema and corneal endothelial abnormalities, prior to the surgical iridectomy makes this unlikely. Also, the absence of abnormality of corneal endothelium in other family members, together with the normal endothelial layer in the patient's other eye, makes the diagnosis of posterior polymorphous endothelial dystrophy highly improbably.

Since the early descriptions of Chandler's syndrome and the clinical identification of a spectrum of corneal and iris abnormalities in the iridocorneal endothelial (ICE) syndrome, the etiology of the endothelial layer abnormalities and iris changes has been the subject of much contention. Recent evi-
Fig. 6. Scanning electron micrograph of posterior corneal cell layer, showing prominent microvillous projections at cell boundaries and over the surface of paler cells, except for a "nuclear" bare area. A second cell-type, apparently darker, has far fewer microvillous projections (x1000).

dence suggests that the primary defect in Chandler's syndrome (and in the ICE syndrome) is an abnormal posterior corneal cell layer, which migrates across the trabecular meshwork and iris, causing the iris defects and glaucoma. Although the site of origin of these migrating cells appears definitely to be the posterior corneal surface, their phylogenetic origin is still conjectural. It has been suggested that these may, in fact, be mesothelially undifferentiated cells, or they may resemble fibroblasts with an increased rough-surface intracytoplasmic reticulum and junctional complexes with microvillous projections. The possibility that these cells resemble epithelial cells has been suggested by Richardson. In fact, the ultrastructural descriptions of the posterior cornea of a number of the reported cases include intracytoplasmic filaments and microvillous projections, although the morphology of these cells as epithelial-like has not been substantiated. In fact, in contradistinction to the cells lining the posterior corneal surface in posterior polymorphous endothelial dystrophy, Rodrigues et al make the point that the cells lining the posterior cornea in Chandler's syndrome have no epithelial-like characteristics.

The presence of ultrastructural features of increased intercellular digitations, microvillous surface projections, bundles of 10-nm intracytoplasmic filaments, desmosomal formation, and production of basement membrane and collagen could indicate that these cells have an epithelial origin or nature. Unlike the cells in posterior polymorphous endothelial dystrophy or of epithelial ingrowth, in the present patient they did not form more than two layers on the cornea or more than a monolayer on the iris. The prominent and unequivocal staining, with rabbit antikeratin antibody, of the cells lining the endothelial surface as compared to the nonstaining of the control cornea supports the contention that these cells are epithelial in nature. Why this staining of corneas from other Chandler's syndrome cases proved negative for keratin remains an enigma. This method has been effectively used to demonstrate the presence of keratin in posterior polymorphous dystrophy.

The results of specular microscopy, as described previously in grade 3 of the ICE syndrome, are similar to the results in the present case, with prominent light-dark "reversal" of cell outlines and large central areas that are devoid of detail. The specular microscopic morphology is mainly related to the cell boundary interface with the aqueous humor, and this is affected by changes in refraction at this surface. Scanning electron microscopy of this same posterior corneal surface disclosed that the most evident abnormality (as compared to a normal endothelial layer) was the varying density of the microvillous projections, which were prominent in this patient. The major density was around the periphery of the cell, with nuclear-like projections present centrally, which had only a few microvillous projections. This correlates very well with the specular microscopic appearance, with the cell outlines being demarcated, and with the central area (nearly devoid of microvillous projections) being dark.

Our antikeratin antibody staining results confirmed the epithelial-like characteristics previously suspected by some ultrastructural examinations. We also correlated the scanning electron microscopy and specular microscopy of the endothelial layer of this cornea of a patient with Chandler's syndrome. The specular microscopic morphology and appear-
Fig. 7. Rabbit antikeratin antibody staining of control cornea (A, B, C) and of Chandler's syndrome cornea (D, E, F). A and D reveal no fluorescence with staining by rabbit preimmune serum (X80). B shows epithelial fluorescence with no endothelial fluorescence (double arrowheads) in the control cornea, with rabbit antikeratin antibody staining. E shows strong epithelial and endothelial fluorescence (double arrowheads) in the Chandler's syndrome cornea with rabbit antikeratin antibody staining. C and F show the difference in endothelial fluorescence of control cornea vs Chandler's syndrome corneas, at higher magnification (X220).
ance can now be understood in terms of the microvillous projections present on the aqueous-humor border of the cell membrane.

No explanation has been forthcoming as to why human endothelial cells, which are noteworthy for their inability to mitose, should migrate off of the cornea.

Whether the epithelial characteristics of the “endothelium” in Chandler’s syndrome represent a congenital variant (as in posterior polymorphous dystrophy) or are an acquired change in the endothelium is unknown. The fact that corneal endothelium can sometimes develop characteristics of fibrocytes and can produce collagen seems well established.16,17 Also, the fact that cells on the posterior corneal surface were epithelial-like in the present case of Chandler’s syndrome may offer an explanation of this unusual in vivo behavior of these cells lining the posterior corneal surface, and also the relative impermeability of these corneas to fluorescein.18 These findings should prompt a closer examination of other cases of Chandler’s syndrome, to confirm whether or not this condition is an epithelialization disease.

**Key words:** Chandler’s syndrome, specular microscopy, corneal endothelium, ultrastructure, epithelial cells, keratin

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**References**