**Endothelial Metaplasia in the Iridocorneal Endothelial Syndrome**

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**Purpose.** To test the hypothesis that the aberrant, cytokeratin-expressing cells that replace endothelium in the iridocorneal endothelial (ICE) syndrome are of endothelial origin.

**Methods.** Corneas from four patients with Chandler’s syndrome and three with essential iris atrophy were examined by two-color immunofluorescence for simultaneous expression of cytokeratins and two markers of endothelial lineage: vimentin and the antigen recognized by the antihuman monoclonal antibody 2B4.14.1.

**Results.** In six corneas, unequivocal endothelial staining for cytokeratins was present; in each of these, cells coexpressing cytokeratins and the two endothelial markers were clearly identifiable. In the remaining cornea, weak cytokeratin staining that colocalized with vimentin was present.

**Conclusions.** These results lend strong support to the hypothesis that the “epithelial-like” endothelial cells in ICE syndrome are cells of endothelial lineage rather than heterotopia of epithelial cells; these cells probably arise via a metaplastic transformation of preexisting endothelium. Invest Ophthalmol Vis Sci. 1997;38:1896–1901.

The iridocorneal endothelial (ICE) syndrome is a group of progressive ocular disorders whose common features encompass abnormalities of the cornea, iris, and iridocorneal angle.† Included in the spectrum of ICE syndrome are Chandler’s syndrome, a disorder characterized by corneal edema, glaucoma, and iris and angle changes; iris nevus (Cogan–Reese) syndrome, in which the iridocorneal abnormalities include cellular nodules on the anterior surface of the iris; and essential iris atrophy, in which full-thickness iris atrophy is superimposed on abnormalities similar to those in Chandler’s syndrome.

Many of the clinical manifestations of ICE syndrome are attributable to pathologic alterations of the...
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Asian in one. The age range was 30 to 76 years, with patients. The race was white in five, black in one, and seven patients with ICE syndrome—four with Chandler's syndrome and three with essential iris atrophy—were included in the study. The ocular process was unilateral in all patients, and there was no history of ocular disease in any family members of the seven patients. The race was white in five, black in one, and Asian in one. The age range was 30 to 76 years, with an average of 52 years. Six of the seven patients were older than 45 years. The left eye was involved in four and the right in three patients. All studies were performed in accordance with the tenets of the Declaration of Helsinki and with approval of the responsible institutional human experimentation committee; informed consent was obtained before all diagnostic and therapeutic procedures.

The specimens included six corneal buttons removed at penetrating keratoplasty and one globe. Donor corneal rims from two of the keratoplasties were also included in the study as normal controls. Tissues were fixed in a phosphate-buffered solution containing 3% formaldehyde and 1% glutaraldehyde and were embedded in paraffin. In all cases, routine histologic sections stained by the hematoxylin and eosin method were examined by one of us.

Monoclonal Antibodies. 2B4.14.1 (anticorneal endothelium, IgM)10 was produced from the hybridoma cell line, generously provided by Dr. Fred Sanfilippo (Johns Hopkins University, Baltimore, MD). 2B4.14.1 reacts strongly with corneal endothelium but does not stain epithelial cells or stromal keratocytes. Relatively uniform endothelial staining for 2B4.14.1 has been observed in various corneal disorders, including keratoconus, Fuchs' dystrophy, lattice corneal dystrophy, pseudophakic bullous keratopathy, and herpetic keratitis (DN Howell and FP Sanfilippo, unpublished observations, 1991). AE1/AE3 (a mixture of two anticytokeratin monoclonal antibodies, both IgG1) and Vim 3B4 (antivimentin, IgG2a) were purchased from Dako Corporation (Carpinteria, CA).

Immunofluorescent Staining. Sections with a thickness of 4 μm were cut, placed on positively charged glass slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA), deparaffinized, and rehydrated through a series of graded ethanol. Glutaraldehyde-induced autofluorescence was abrogated by incubation for 30 minutes at 25°C in 0.1% sodium borohydrate in phosphate-buffered saline (PBS) with a pH of 7.4.11 After three washes with PBS, the sections were incubated for 10 minutes at 37°C with 0.25% pepsin with a pH of 2.0 (Sigma Chemical, St. Louis, MO) to unmask antigenic sites reactive with the anticytokeratin and antivimentin monoclonal antibodies. Sections were washed three times with PBS and then incubated for 10 minutes at 25°C with 1% bovine serum albumin in PBS to decrease nonspecific reagent binding.

Simultaneous detection of cytokeratins and 2B4.14.1 antigen was accomplished as described previously.8 Briefly, sections were incubated for 30 minutes at 25°C with a mixture of AE1/AE3 (IgG1) and 2B4.14.1 (IgM). In all experiments, serial sections were stained with isotype-matched negative control primary antibodies to ensure the specificity of the observed staining. After three washes with PBS, the sec-
TABLE I. Compendium of Endothelial Two-Color Immunostaining Results

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Diagnosis</th>
<th>Cytokeratin Staining</th>
<th>2B4.14.1/Cytokeratin</th>
<th>Vimentin/Cytokeratin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chandler’s</td>
<td>Focal</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Chandler’s</td>
<td>Diffuse</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Chandler’s</td>
<td>Diffuse</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Chandler’s</td>
<td>Diffuse</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>EIA</td>
<td>Focal*</td>
<td>-</td>
<td>+/-*</td>
</tr>
<tr>
<td>6</td>
<td>EIA</td>
<td>Focal</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>EIA</td>
<td>Diffuse†</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Chandler’s = Chandler’s syndrome; EIA = essential iris atrophy.
* In patient 5, weak positive cytokeratin staining was seen in the cytokeratin-vimentin double stain.
† In patient 7, only a small amount of endothelium remained, but all remaining cells expressed cytokeratins.

RESULTS. Unequivocal endothelial staining for cytokeratins was detected in four of four patients with Chandler’s syndrome and two of three patients with essential iris atrophy. In one additional patient with essential iris atrophy, weak, variable cytokeratin staining was detected. In all patients, two-color immunofluorescence revealed co-localization of cytokeratins with 2B4.14.1 antigen, vimentin, or both (Table 1).

In four patients, including three with Chandler’s syndrome (patients 2, 3, and 4) and one of essential iris atrophy (patient 7), the majority of the endothelial cells stained for all of the antigens (Fig. 1A, cytokeratin–2B4.14.1 double stain, and Fig. 1B, cytokeratin–vimentin double stain). In each panel, the top strip shows cytokeratin staining (green) and the middle strip shows staining for the endothelial marker (red) in the same microscopic field. The bottom strips are green-red double exposures, in which areas of co-localization of cytokeratins and the endothelial markers appear yellow. In many areas, a thin line of 2B4.14.1 (red) staining without associated cytokeratins was present.
ent superficial to the zone of cytokeratin−2B4.14.1 colocalization (yellow) (Fig. 1A, bottom strip). This observation may reflect expression of the 2B4.14.1 antigen on the cell surface, an area presumably devoid of cytokeratins.\(^8\)

In one additional patient with Chandler’s syndrome (patient 1; Figs. 1C, 1D) and one with essential iris atrophy (patient 6; Figs. 1E, 1F), cytokeratin staining was more patchy. In these patients, double staining for cytokeratins and 2B4.14.1 antigen revealed a mixture of cells expressing both antigens and cells expressing only one or the other (Figs. 1C, 1E). The results of cytokeratin−vimentin double stains (Figs. 1D, 1F) were similar.

In one patient with essential iris atrophy (patient 5), weak cytokeratin staining was seen in a minority of endothelial cells on cytokeratin−vimentin double stains; these cells also expressed vimentin (data not shown). Cytokeratin reactivity was not detected on cytokeratin−2B4.14.1 double stains in this patient. This discrepancy may reflect either sampling error or greater sensitivity of the secondary antibody detection system used in the cytokeratin−vimentin double stain.

Staining of two normal donor corneal rims revealed the expected pattern of endothelial staining, with uniform expression of 2B4.14.1 antigen and vimentin and absence of cytokeratins (data not shown). As expected, stromal keratocytes in all patients stained for vimentin but did not express 2B4.14.1 antigen or cytokeratins (Fig. 1). Epithelial cells in all patients also exhibited the expected phenotype, staining strongly for cytokeratins but failing to express 2B4.14.1 antigen or vimentin (data not shown).

**DISCUSSION.** The findings of this investigation confirm and extend those of previous studies that document the presence of endothelial cytokeratins in corneas from patients with ICE syndrome.\(^1–4\) Specifically, our experiments indicate that a majority of the endothelial cells that contain cytokeratins also express 2B4.14.1 antigen and vimentin, two markers expressed by normal endothelial cells but absent from normal epithelium. (A single conflicting report of coexpression of cytokeratins and vimentin by both normal corneal endothelium and epithelium\(^5\) is not supported by our findings or those of any of the other studies cited herein.)

Our results provide strong support for the hypothesis that the cytokeratin-positive epithelial-like cells in ICE syndrome are of endothelial lineage. In this regard, ICE syndrome resembles PPD.\(^6\) The endothelial cytokeratin expression in ICE syndrome, like that in PPD, occurs in several patterns; cytokeratin-positive cells can be present in groupings ranging from small, irregular patches to large, confluent sheets. This variability could reflect either disparate disease subtypes or variable degrees of disease progression. In our limited sample, the pattern variability did not appear to correlate with recognized clinicopathologic subtypes of ICE syndrome; examples of both diffuse and focal cytokeratin staining patterns were seen in corneas from patients with both Chandler’s syndrome and essential iris atrophy.

In contrast, our findings mitigate against the alternative hypothesis that the epithelial-like cells in ICE syndrome represent embryonic rests or heterotopia of true epithelial cells. Such cells would not be expected to express 2B4.14.1 antigen or vimentin. Although it is theoretically possible that epithelial heterotopia in an endothelial milieu could acquire endothelial characteristics through differentiation, the simultaneous acquisition of two totally distinct markers of endothelial lineage (one a cytoplasmic intermediate filament and the other a cell-surface molecule) is highly improbable. Also, although our experiments detected occasional cytokeratin-positive, 2B4.14.1 antigen-negative cells, cytokeratin-positive, vimentin-negative cells were rarely seen.

The presence of cytokeratin-positive endothelial cells can be explained by three potential pathogenetic sequences. Such cells could be present congenitally, could arise by proliferative overgrowth of normal endothelium by progeny of a limited number of aberrant progenitor cells, or could develop by metaplastic transformation of preexisting endothelium. Although our findings do not provide direct evidence for or against any of these possibilities, the first two are considered unlikely. A congenital origin for the cytokeratin-positive cells is at variance with the current concept of ICE syndrome as an acquired disorder.\(^13,14\) The cell-proliferation hypothesis fits poorly with specular microscopy observations; although occasional patients have regional corneal involvement by expanding patches of cells that might be interpreted as clusters of proliferating abnormal endothelium,\(^15\) most have diffuse, progressive alterations in endothelial cell size and shape.\(^16\) The latter pattern is more consistent with a metaplastic alteration of preexisting endothelium.

Because keratoplasty specimens represent single (and generally late) time points in the progression of corneal disease, it is impossible to pinpoint the onset of endothelial cytokeratin expression. Although it is tempting to speculate that cytokeratin production parallels the clinical progression of ICE syndrome, it is also possible that it antedates the development of clinical symptoms by months or years. Alternatively, it could occur at a relatively late stage in a series of events leading to endothelial dysfunction.

The mechanism leading to cytokeratin expression by corneal endothelium is also unclear. Endothelial
cytokeratins occur in both PPD and ICE syndrome, two disease complexes that share certain clinical features but appear to differ in pathogenesis. PPD is a heritable disorder that is often bilateral in expression. In contrast, the diseases that constitute ICE syndrome appear to be acquired, and disease expression is most often unilateral. In one study, herpes simplex virus genomic material was detected in a majority of corneas from patients with ICE syndrome, raising the possibility that the syndrome has an infectious etiology. We also recently detected coexpression of cytokeratins and 2B4.1 antigen in a cornea from a patient with Fuchs' dystrophy (T Damms and DN Howell, unpublished observations, 1996). Taken together, these findings suggest that cytokeratin production may be a final common pathway for a variety of pathologic processes affecting corneal endothelium.

**Key Words**

Corneal endothelium, cytokeratins, immunofluorescence, iridocorneal endothelial syndrome, metaplasia

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**References**