The immunocytologic characteristics of two formalin-fixed, paraffin-embedded corneas from patients with the iridocorneal endothelial (ICE) syndrome and unaffected control corneas were studied. Binding of polyclonal antisera to Factor VIII, S-100 protein, involucrin, neuron specific enolase (NSE), and the lectins peanut agglutinin and Ulex europaeus agglutinin-I was performed using the standard peroxidase-anti-peroxidase method. We detected reactive patterns of monoclonal antibodies to cytokeratins (34BE12 is a 56–58 kDa mouse IgG reactive to stratified epithelia; Pkk1 is a 44–54 kDa mouse IgG reactive to simple epithelia; and KL1 is a 55–57 kDa mouse IgG reactive to epidermis and simple epithelia) using the standard avidin-biotin complex method. Staining properties were similar for the polyclonal antisera, lectins, NSE, and chromogranin in corneas with ICE syndrome and in the controls. However, the cytokeratins 34BE12, Pkk1, and KL1 were detected in the endothelium of the corneas with the ICE syndrome but not in the controls. These findings suggest that various cytokeratins are expressed in the corneal endothelium in the ICE syndrome that are not expressed in unaffected corneal endothelium. Invest Ophthalmol Vis Sci 33:3581–3585, 1992

Iris nevus syndrome, Chandler’s syndrome, and essential iris atrophy are characterized by unilateral glaucoma and peripheral anterior synchiae that develops in a previously open anterior chamber angle and occurs mainly in young to middle-aged women. These three diseases probably are variants seen in the spectrum of the iridocorneal endothelial (ICE) syndrome. Interestingly, the acronym ICE also stands for the three clinical disorders. One previous study emphasized the epithelial-like characteristics of endothelial cells in the ICE syndrome.1

To further characterize the pathologic features of corneal endothelium in the ICE syndrome, we evaluated the immunohistochemical features of two corneas from patients with the ICE syndrome and two control corneas. Monoclonal and polyclonal serum reactive to neuroectodermal, neuroendocrine, and epithelial markers were evaluated. These antisera were chosen because the endothelium is of neural crest origin and the endothelium in the ICE syndrome has epithelial-like characteristics, as described above.

Materials and Methods

Corneal specimens obtained at penetrating keratoplasty from two patients with the clinical diagnosis of iridocorneal endothelial (ICE) syndrome and two age-matched normal control corneas obtained from the Georgia Lions Eye Bank, Atlanta, were submitted in 10% formalin and bisected. One half from each corneal button was processed routinely for light microscopy and immunohistochemistry using the standard avidin-biotin complex2 or peroxidase-anti-peroxidase3 technique. Primary commercially available antisera were to cytokeratins 34BE12, KL1, and Pkk1; chromogranin; factor VIII-related antigen; S-100 protein; involucrin; neuron-specific enolase (NSE); Ulex europaeus agglutinin; and peanut agglutinin. The remaining half from each corneal button was processed routinely for scanning and transmission electron microscopy.

Results

Examination of both specimens by light microscopy showed findings typical for the iridocorneal endothelial syndrome. Examination of both specimens by scanning electron microscopy revealed findings
typical for the ICE syndrome, including an abnormal endothelial mosaic and numerous microvillus processes projecting posteriorly (Fig. 1). Transmission electron microscopy of the specimens showed that Descemet's membrane had normal banded embryonic (1.1 μm) and homogenous postnatal (4.5 μm) layers. Also, a thin posterior collagenous layer (0.3 μm) was present. Most of the endothelial cells were elongated and rectangular with boxcar nuclei, abundant intracytoplasmic mitochondria, intracytoplasmic pigment granules, and prominent cytoplasmic processes (Fig. 2). Some endothelial cells had irregular nuclei and fusiform shapes. A few cells had prominent cytoplasmic extensions that contained numerous intracytoplasmic tonofilaments. In some areas, there was a double layer of endothelial cells. Results of the immunohistochemical staining are summarized in Table 1.

The cytokeratins 34BE12, Pk1, and KL1 were expressed in the endothelium of the corneas with the ICE syndrome but not the controls (Fig. 3). These three cytokeratins also were expressed in the epithelium of the corneas with ICE syndrome and the controls. The lectin Ulex europeus was positive in the basal layers of the epithelium of corneas from the ICE syndrome patients and throughout all epithelial layers in the controls. The endothelium was focally positive in the ICE syndrome corneas and in the controls. The lectin peanut agglutinin was negative in the epithelium and in the endothelium in the corneas from the patients with the ICE syndrome and in the controls. Although formalin fixation theoretically may have affected interpretation of antigenic expression, this effect was minimal because the sensitivity and specificity of the antisera had been tested by the manufacturers for formalin-fixed tissues, and appropriate formalin-fixed controls were examined.

Discussion

One previous study showed keratin positivity in normal corneal endothelium using a cocktail of cytokeratins.4 Other studies5-7 failed to demonstrate cytokeratin staining in normal corneal endothelium. One of these studies6 also found cytokeratin positivity in corneal endothelial cells in posterior polymorphous dystrophy and correlated the cytokeratins with intracytoplasmic tonofilaments. Our findings agree with the studies that showed lack of cytokeratin positivity in normal corneal endothelium.5-7 Our study differs from a previous study7 that failed to show endothelial staining for a keratin cocktail in the ICE syndrome. However, our study agrees with Hirst and coworkers,1 who found positive indirect immunofluorescent staining for cytokeratins AE1, AE3, and AE4 in the endothelium of a corneal button obtained from a patient with Chandler's syndrome. The difference in detection of cytokeratins in endothelial cells in ICE syndrome may reflect a variability of cytokeratin expression in endothelial cells or a difference in the cytokeratins analyzed. It also is possible that in our second case, the mechanical effects from previous surgery influenced cytokeratin expression. A previous case originally published as Chandler's syndrome8 later was determined to be posterior polymorphous dystrophy, a condition in which the corneal endothelium may express cytokeratins.6 It is possible that either of our cases may be determined later to be posterior polymorphous dystrophy, but we believe this is unlikely because the patients have been followed for several years without evidence of contralateral corneal disease.

Involucrin is present only in squamous epithelia, and its presence in mucous membranes has been re-
Fig. 2. Examination by transmission electron microscopy shows fibrillar collagenous material (between arrowheads) between the post-natal homogenous portion of Descemet's membrane (D)—which is studded with figures of 110 nm banded material (open arrows)—and multilayered endothelium. The endothelium contains intracytoplasmic pigment granules (p), aggregates of tonofilaments (tf), and posterior microvilli (arrows). (×18,000, inset ×84,100.)

ported only when associated with metaplastic, dysplastic, or neoplastic processes. Involucrin was positive only in the epithelium of the second ICE syndrome cornea. The only lectin detected in the corneas was Ulex europaeus agglutinin (UEA-1). Our results are consistent with previous studies in that UEA-1 was present and peanut agglutinin, the expression of which generally is more variable, was absent. Neither the controls nor the ICE syndrome corneas stained for neuronal (NSE, S-100), neuroendocrine (NSE, chromogranin), neuroectoderm (S-100, NSE) or glial cell (S-100) markers. The vascular endothelial marker

Table 1. Immunohistochemical findings for ICE and control corneas

<table>
<thead>
<tr>
<th>Antibody/lectin</th>
<th>ICE Specimen no. 1</th>
<th>ICE specimen no. 2</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epi</td>
<td>Endo</td>
<td>Epi</td>
</tr>
<tr>
<td>Involucrin</td>
<td>–</td>
<td>–</td>
<td>+*</td>
</tr>
<tr>
<td>NSE</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>UEA-1</td>
<td>+*</td>
<td>+</td>
<td>+*</td>
</tr>
<tr>
<td>PNA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>S100</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>34BE12</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pkk1</td>
<td>+</td>
<td>+†</td>
<td>+</td>
</tr>
<tr>
<td>KL1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Stain was positive only in the suprabasal surface epithelial layers.
† Stain was positive only focally in the endothelium.
epi, epithelium. endo, endothelium. –, no staining. +, staining. NSE, neuronal specific enolase. UEA-1, Ulex europaeus. PNA, peanut agglutinin. 34BE12, Pkk1, and KL1 are cytokeratins. Stromal cells failed to stain with any antibody or lectin.
Fig. 3. (A) There is positive intracytoplasmic staining for cytokeratins Pkk 1 in the epithelium and endothelium of a cornea with the ICE syndrome (case 2). (X80.) (B) The endothelium strongly stains for cytokeratins Pkk 1 in a cornea with the ICE syndrome (case 2). (X250.) (C) The epithelium in a control cornea stains for and the endothelium fails to stain for cytokeratins Pkk 1. (X40.) (D) Close inspection shows a lack of staining in a control corneal endothelium for cytokeratins Pkk 1. (X250.)

(Factor VIII) was not detected in the endothelium of the corneas from the controls or the ICE syndrome patients. This agrees with a previous study. 4

We propose that although normal corneal endothelium may express cytokeratins, this expression is enhanced in the ICE syndrome and posterior polymorphous dystrophy, 6 with both conditions showing epithelial-like morphologic changes in the corneal endothelium. There are important differences between posterior polymorphous dystrophy and the ICE syndrome. Posterior polymorphous dystrophy is a congenital, bilateral, inherited disease with Descemet's membrane abnormalities occurring in utero or shortly after birth. In this case, poor embryonic differentiation of the endothelium results in endothelial cytokeratin expression. 6 On the other hand, the ICE syndrome is an acquired, unilateral condition with Descemet's membrane abnormalities that occur during adulthood. In this case, a metaplastic process in the endothelium results in cytokeratin expression.

Key words: corneal endothelium, cytokeratins, iridocorneal endothelial (ICE) syndrome

References

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