Extracellular Matrix Alterations in Human Corneas With Bullous Keratopathy

Alexander V. Ljubimov,* Robert E. Burgeson,† Ralph J. Butkowski,‡ John R. Couchman,§ Rong Rong Wu,§ Yoshifumi Ninomiya,‖ Yoshikazu Sado,‖ Ezra Maguen,* Anthony B. Nesburn,* and M. Cristina Kenney*

**Purpose.** To uncover abnormalities of extracellular matrix (ECM) distribution in human corneas with pseudophakic and aphakic bullous keratopathy (PBK/ABK).

**Methods.** Indirect immunofluorescence with antibodies to 27 ECM components was used on frozen sections of 14 normal and 20 PBK/ABK corneas.

**Results.** Fibrillar deposits of an antiadhesive glycoprotein tenascin in the anterior and posterior stroma, epithelial basement membrane (BM), bullae and subepithelial fibrosis (SEF) areas, and posterior collagenous layer (PCL) were revealed in diseased corneas. Tenascin in midstroma, which was observed in some cases, correlated with decreased visual acuity. In normal central corneas, tenascin was never found. Other major ECM abnormalities in PBK/ABK corneas compared to normals included: discontinuous epithelial BM staining for laminin-1 (a1b1y1), entactin/nidogen and fibronectin; accumulation of fibronectin and a1–a2 type IV collagen on the endothelial face of the Descemet’s membrane; and abnormal deposition of stromal ECM (tenasin, fibroectin, decorin, types I, III, V, VI, VIII, XII, XIV collagen) and BM components (type IV collagen, perlecacn, bamacan, laminin-1, entactin–nidogen, fibronectin) in SEF areas and in PCL.

**Conclusions.** This study provides a molecular description of an ongoing fibrosis on the epithelial, stromal, and endothelial levels in PBK/ABK corneas. These fibrotic changes may follow initial endothelial damage after cataract surgery, may be caused by the upregulation of fibrogenic cytokines, and may play a significant role in the progression of bullous keratopathy.

surgery, to an alteration of the pumping capacity of these cells, or both. The damaged endothelium can close the initial defect over time, but, in some cases, the defect persists, leading to the development of PBK/ABK. As a potential therapy, transplantation of normal corneal endothelium onto its basement membrane (DM) has been considered. However, normal endothelium grows well on normal DM but does not spread and grow well on PBK DM. The diseased DM thus provides a poor substrate for the cells. A reduction of endothelial cell number may be an important early event in the PBK/ABK development. Later, changes of the cell phenotype may occur, leading to the formation of a growth-restricting extracellular matrix (ECM) that precludes endothelial reestablishment.

Endothelial cell dysfunction also may contribute to corneal edema in PBK/ABK. These cells maintain corneal hydration through the Na⁺/K⁺ ATPase pump. Corneal endothelial cells in PBK/ABK have reduced pump site density compared to normals. This functional impairment may be a secondary event in PBK/ABK.

Several lines of evidence emphasize the importance of ECM changes in PBK/ABK development:

1. Typically, PBK/ABK corneas develop ECM accumulation at the endothelial (PCL) and the epithelial (SEF) levels.
2. Corneal epithelial basement membrane (BM) in PBK/ABK lacks fibronectin, laminin, and type IV collagen, which might reduce cell adhesion and facilitate bullae formation.
3. PBK DM shows altered collagen content and lectin binding, and, contrary to normal DM, does not support cell growth, suggesting structural alterations, compositional alterations, or both.

Despite these findings, the ECM of PBK/ABK corneas has not been studied systematically.

We report here on the detailed characterization of the ECM and BM composition of PBK/ABK corneas with antibodies to 27 individual ECM components. Compared to normal corneas, there was an accumulation of stromal tenasin and alterations of epithelial BM and DM. We also describe the molecular and cellular composition of PCL and SEF areas. Our results provide the documentation of significant fibrotic changes in PBK/ABK corneas on the molecular level, even in cases in which no PCL or SEF could be found. We think PBK/ABK not only is a disease of chronic edema but has an ongoing fibrosis that plays an important role in its pathology.

MATERIALS AND METHODS

Normal adult human corneas (n = 14) were obtained within 36 hours of death from the National Disease Research Interchange (Philadelphia, PA). PBK/ABK corneas (n = 20, Table 1) were obtained within 20 hours of penetrating keratoplasty. In each case, the histologic diagnosis was verified by a pathologist. Corneal embedding in OCT compound (Miles, Elkhart, IN), sectioning, indirect immunofluorescence, and photography were performed as described. Results of routine specificity controls were negative. Statistical analysis was performed using two-sided Fisher's exact test.

Antibodies to human α1-α6 chains of type IV collagen; to α1 (A), α2 (M), α3 (K), β1 (B1), β2 (S), β3 (kalinin B1), and γ1 (B2) chains of laminin; to entactin/nidogen, types VII, XII, and XIV collagen, perlecan core protein domain IV (clones A7L6 and C1L11); and to fibronectin have been described in detail. Affinity-purified polyclonal antibodies to recombinant rat bamacan (basement membrane chondroitin sulfate proteoglycan) core protein, cross-reacted with human, were produced in rabbits (Couchman et al, manuscript in preparation). A monoclonal antibody to the α3 chain of human type VI collagen (clone 3C4) was a gift from Dr. E. Engvall (La Jolla Cancer Research Foundation, La Jolla, CA). Polyclonal antibodies to human types I, III, and V collagen were obtained from Southern Biotechnology (Birmingham, AL); polyclonal antibodies to human decorin and a monoclonal antibody to human tenasin (clone TN-2) were obtained from Chemicon International (Temecula, CA); a monoclonal antibody to human vimentin (clone Vim 3B4) was obtained from Boehringer Mannheim (Indianapolis, IN); a monoclonal antibody to cellular fibronectin (clone IST-9) was obtained from Sera-Lab (Crawley Down, UK); a monoclonal antibody to the α1 chain of bovine-human type VIII collagen (clone 9H3) was obtained from Seikagaku America (Rockville, MD); a polyclonal antibody to human von Willebrand factor, a mixture of monoclonal antibodies to human keratins (pankeratin cocktail), and a monoclonal antibody to human α-smooth muscle actin (clone 1A4) were all obtained from Sigma Chemical (St. Louis, MO). Cross-species-adsorbed fluorescein- and rhodamine-conjugated secondary antibodies were obtained from Chemicon International.

RESULTS

All PBK/ABK corneal buttons studied represented only the central corneal part, so their epithelial BM structure was compared with that of normal central corneas only (see for differences of the epithelial BM composition in normal central cornea and limbus). In PBK/ABK corneas and failed corneal grafts of pseudophakic–aphakic eyes (Table 1), similar ECM and
### Extracellular Matrix of Bullous Keratopathy Corneas

#### TABLE 1. PBK/ABK Patient History

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Time Between Cataract Removal and Corneal Transplantation (years)</th>
<th>Diagnosis</th>
<th>Visual Acuity*</th>
<th>Tenascin in Midstroma</th>
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<tr>
<td>1</td>
<td>75</td>
<td>F</td>
<td>2</td>
<td>PBK</td>
<td>HM</td>
<td>±</td>
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<tr>
<td>2</td>
<td>74</td>
<td>M</td>
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<td>+</td>
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<tr>
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<td>F</td>
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<td>CF</td>
<td>±</td>
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<tr>
<td>6</td>
<td>69</td>
<td>M</td>
<td>3</td>
<td>PBK</td>
<td>CF</td>
<td>±</td>
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<tr>
<td>7</td>
<td>82</td>
<td>F</td>
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<td>PBK</td>
<td>CF</td>
<td>±</td>
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<td>8</td>
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<td>CF</td>
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<td>ABK</td>
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<td>±</td>
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<td>79</td>
<td>M</td>
<td>5</td>
<td>Failed graft, aphakic</td>
<td>CF</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>89</td>
<td>F</td>
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<td>ABK</td>
<td>CF</td>
<td>±</td>
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<td>200/400</td>
<td>-</td>
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<tr>
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<td>81</td>
<td>F</td>
<td>4</td>
<td>PBK</td>
<td>200/100</td>
<td>-</td>
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<td>16</td>
<td>57</td>
<td>F</td>
<td>12</td>
<td>PBK</td>
<td>20/50</td>
<td>-</td>
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<tr>
<td>17</td>
<td>57</td>
<td>M</td>
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<td>20/60</td>
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<td>18</td>
<td>79</td>
<td>F</td>
<td>3</td>
<td>PBK</td>
<td>200/400</td>
<td>-</td>
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<tr>
<td>19</td>
<td>68</td>
<td>M</td>
<td>6</td>
<td>PBK</td>
<td>200/100</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>78</td>
<td>M</td>
<td>10</td>
<td>PBK</td>
<td>20/150</td>
<td>-</td>
</tr>
</tbody>
</table>

HM = patient recognizes hand movements; CF = patient can count fingers; + = present; − = absent; ± = local and/or weak expression.

* Before last corneal transplantation.

† Time between last two consecutive corneal transplantations.

The patient was aphakic for 8 years and then pseudophakic for 11 years.

BM changes were observed; therefore, the data on these two groups were combined.

#### Tenascin

Normal central corneal epithelial BM, stroma, and DM were negative for tenascin (Figs. 1A, 1C). In contrast, all PBK/ABK corneas had fibrillar tenascin deposits in the anterior stroma beneath Bowman’s layer (Fig. 1B) and in the posterior stroma adjacent to the DM (Fig. 1D). Tenascin also was present in parts of epithelial BM and the DM (not shown), in areas of bullae formation (Fig. 1B), SEF, and PCL (Fig. 1D). In some cases, usually more advanced (Table 1), tenasin deposits were seen in the midstroma as well. It should be noted that in normal limbus, tenasin was found in the stroma and only in a part of limbal blood vessels (not shown). This vessel staining pattern is similar to that observed in other tissues for tenasin-C but differs from that of another tenasin variant, tenasin-X, always associated with blood vessels. These data suggest that our antibody recognized the ubiquitous tenasin variant, tenasin-C.

#### Type IV Collagen Chains

In all PBK/ABK corneas, the staining for α1(IV) and α2(IV) chains was observed on the endothelial face of the DM, often together with the staining of the stromal face (Fig. 2B). This was in contrast to normal age-matched corneas in which these chains usually were found on the stromal face of the DM (Fig. 2A). In more than half of PBK/ABK corneas, the epithelial BM was partially positive for these chains, a pattern that was never seen in normal central corneas (not shown). The distribution of the α3(IV)–α6(IV) chains was similar to that in normal corneas, with infrequent local discontinuities of the epithelial BM (not shown).

Using a new panel of monoclonal antibodies to all six α(IV) chains, we have expanded our recent results on the heterogeneity of α(IV) chain distribution in the epithelial BM of central cornea, limbus, and conjunctiva. Both the α5(IV) and the α6(IV) chains were found in the central, limbal, and conjunctival [weaker α5(W) staining than in the limbus] regions of epithelial BM. Staining for α3(IV) and α4(IV) chains was continuous in the central cornea and discontinuous or absent in the limbus and proximal conjunctiva, but it could reappear in distal conjunctiva on sections denatured in 6 M urea. In the DM, α6(IV) chain was seen on the endothelial face, as were α3(IV)–α5(IV) chains. Unlike the latter three chains, α6(IV) chain was not found around keratocytes.

#### Fibronectin

The PBK/ABK corneas had local fibrillar accumulations of fibronectin (including cellular isoform) in the...
FIGURE 1. Tenascin in normal and PBK/ABK corneas. No staining is seen in normal corneas in the anterior (A) and posterior (C) parts. In PBK/ABK corneas, tenascin fibrils are found in the anterior stroma and beneath epithelial cells in a bulla (B), as well as in the posterior stroma, on the endothelial face of the DM, and in the PCL (D). E = epithelium; S = stroma; B = Bowman's layer; DM = Descemet's membrane; P = posterior collagenous layer. (arrowhead) Space between detaching epithelium and Bowman's layer in a bulla area. Bar = 40 μm.

anterior stroma (Table 2). The stromal DM face was negative, but the endothelial face was positive (Fig. 2D), unlike the normals that displayed a reverse staining pattern (Fig. 2C). The PBK/ABK epithelial BM showed discontinuous staining (not shown); SEF areas (Fig. 4F) and PCL (Fig. 2D) were positive.

Laminin Chains
The PBK/ABK corneas (Table 2) displayed much weaker than normal and often discontinuous staining of the epithelial BM for laminin-1 (α1/β1γ1, or A-B1-B2) chains (Figs. 2E, 2F). In contrast to this, laminin-5 (kalinin–nicein) was preserved in the epithelial BM of most PBK/ABK corneas, as was another anchoring fibril protein, type VII collagen (not shown). Sparse deposits of laminin-1 were seen in SEF areas (Fig. 4E) and in the PCL (not shown). In the DM, laminin-1 had normal distribution. Laminin chains α2 (M) and β2 (S), normally absent from central corneal epithelial BM, did not appear in PBK/ABK corneas (not shown).

Entactin/Nidogen
Entactin/nidogen staining in PBK/ABK (not shown) was similar to that for laminin-1—that is, it was discontinuous and often absent from the epithelial BM and remained normal in the DM (Table 2). SEF areas and PCL (Fig. 5C) contained some entactin/nidogen.
Extracellular Matrix of Bullous Keratopathy Corneas

### TABLE 2. Major ECM Abnormalities in PBK/ABK Corneas

<table>
<thead>
<tr>
<th>ECM Component</th>
<th>Epithelial BM</th>
<th>Stromal ECM</th>
<th>DM</th>
<th>Subepithelial Fibrosis</th>
<th>PCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenascin</td>
<td>Partially positive</td>
<td>Anterior and posterior deposits†</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>α1-α2 type IV collagen</td>
<td>Partially positive</td>
<td>Anterior deposits</td>
<td>Endothelial face positive</td>
<td>Stromal face mostly negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Partially negative</td>
<td>Anterior deposits</td>
<td>No change*</td>
<td>Mostly negative</td>
<td>Weakly positive</td>
</tr>
<tr>
<td>Laminin-1</td>
<td>Partially negative</td>
<td>No change*</td>
<td>No change*</td>
<td>Mostly negative</td>
<td>Weakly positive</td>
</tr>
<tr>
<td>Entactin/nidogen</td>
<td>Partially negative</td>
<td>No change*</td>
<td>No change*</td>
<td>Mostly negative</td>
<td>Weakly positive</td>
</tr>
<tr>
<td>Bamacan</td>
<td>No change*</td>
<td>No change*</td>
<td>No change*</td>
<td>Weakly positive</td>
<td>Weakly positive</td>
</tr>
<tr>
<td>Decorin</td>
<td>Occasional positive</td>
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<td>No change*</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
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<td>No change*</td>
<td>No change*</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Type XIV collagen</td>
<td>No change*</td>
<td>No change*</td>
<td>No change*</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**ECM = extracellular matrix; BM = basement membrane; DM = Descemet's membrane; PCL = posterior collagenous layer.**  
* Compared with normal corneas  
† In some more advanced cases, tenascin deposits in midstroma were also found (see Table 1).

### Bamacan

This proteoglycan, revealed by the core protein antibody, was found around keratocytes, endothelial cells (Fig. 3A), and limbal blood vessels in the normal corneas. This was unusual because in other tissues, bamacan exclusively associated with BMs.19 In PBK/ABK corneas, it also was seen on the endothelial face of the DM and in the PCL (Fig. 3B) and was associated with SEF, including the BM of abnormal epithelium (Fig. 4D).

### Decorin

As in rabbit corneas,21 decorin core protein was distributed uniformly in the stroma of normal human corneas (Fig. 3C) and in Bowman’s layer. In diseased corneas, it was seen on the endothelial face of the DM, in the PCL (Fig. 3D), and in SEF areas (not shown). The stromal pattern did not change in PBK/ABK (see also5).

### Type VIII Collagen

In normal central corneas, it was localized at the stromal face of the DM (see also25) and as weakly stained fine fibrils in the anterior stroma. Limbal stroma and some of the blood vessels were positive (not shown). In the diseased corneas, type VIII collagen was seen at the endothelial face of the DM, in SEF areas (not shown), and in PCL (Fig. 5A). Stromal distribution usually did not change (not shown).

### Type XII Collagen

In normal corneas, type XII collagen was found at the level of the epithelial BM, throughout the stroma, on the endothelial face of the DM (Fig. 3E), and less intensely in Bowman’s layer. In diseased corneas, it was seen in SEF areas and in PCL (Figs. 3F, 5E) without changes in the stroma.

### Type XIV Collagen

Only weak and irregular staining around keratocytes was observed in normal central corneas. In the limbus, stroma, blood vessels, and epithelial BM were positive (not shown). In PBK/ABK corneas, however, this collagen was accumulated in SEF areas (not shown) and in PCL (Fig. 5B).

### Correlation Between Clinical Features and Extracellular Matrix Changes

Analysis of case histories showed no correlation between the disease duration and severity (evaluated as a degree of vision loss). As shown in Table 1, patients could be arbitrarily divided into three groups by their best-corrected visual acuity before corneal transplantation: recognizing hand movements (severe vision loss), counting fingers, and having visual acuity of 20/400 or better. For most of the ECM changes (including the presence and cellularity of SEF and/or PCL), there was no correlation with visual acuity decrease. However, the presence of tenascin in midstroma (Table 1) was significantly more frequent in group 1 (4 of 4) compared to group 2 (2 of 9; P = 0.021) and especially to group 3 (0 of 7; P = 0.003). There was also a tendency, though not significant, to an increased incidence of tenascin in midstroma in group 2 compared to group 3. The presence of tenascin in midstroma correlated with visual acuity decrease and...
FIGURE 3. Bamacan (A,B), decorin (C,D), and type XII collagen (E,F) in normal (A,C,E) and diseased (B,D,F) corneas. Note abnormal deposition of bamacan (B), decorin (D), and type XII collagen (F) on the endothelial face of the DM and in the PCL in PBK/ABK corneas. E = epithelium; S = stroma; P = posterior collagenous layer. Bar = 40 μm.

Extracellular Matrix Composition of Subepithelial Fibrosis Areas and Bullae in PBK/ABK Corneas

Bullae are regions of fluid accumulation in PBK/ABK corneas either beneath the epithelium or between the epithelial cells. After the bullae resorb, ECM may be deposited in these areas. A subepithelial fibrocellular material (pannus) disrupting the epithelial BM and Bowman’s layer was noted previously in PBK/ABK corneas. In half the PBK/ABK corneas studied here, abnormal ECM deposits with or without cells were found between the epithelium and the Bowman’s layer. Two types of these subepithelial deposits were distinguished here. The first one may be termed ECM-type deposit because only ECM without cells was present between the epithelium and the Bowman’s layer (Fig. 4A). This type of SEF had abnormal accumulations of fibronectin (including cellular), tenascin, perlecain, decorin, and types I, III, IV (mostly α1-α2 chains), V, VI, VIII, XII, and XIV collagen, with some laminin-1, laminin-5, entactin/nidogen, and bamacan (Figs. 4B to 4E). Staining for stromal components was more pronounced than for most BM components (see Figs. 4B to 4E). The second type of SEF (fibrocellular type) contained cells embedded in the ECM, which was qualitatively the same as in the first type (see Fig. 4F). The cells contained vimentin (Fig. 4G) and α-smooth muscle actin (Fig. 4H), typical for myofibroblasts, but did not stain for keratins (not shown here). These cells may be the actual source of the abnormal subepithelial ECM. In areas of bullae without SEF, the basal surface of detaching epithelium also stained for abnormal ECM (Fig. 1B). Some ECM accumulated between epithelial cells or layers (Figs. 1B, 4D, 4F) in contrast to the normal corneas.

Posterior Collagenous Layer Composition of PBK/ABK Corneas

The PCL often is found in PBK/ABK corneas as an additional fibrous layer between the remnant DM and the endothelial cells. In our series of PBK/ABK, the PCL was observed in 80% of cases, in accordance with previous studies. PCL contained types VI, XII, and XIV collagen, tenascin, fibronectin, decorin, bamacan, perlecain, some laminin-1 and entactin/nidogen (Figs. 1D, 2D, 3B, 3D, 3F, 5A to 5E), in addition to the previously reported types I, III, IV, V, and VIII collagen. The presence of tenascin, type XIV collagen, bamacan, and decorin may be significant because they were not seen in normal DM but appeared in diseased DM and in the PCL. PCL also contained α1–α2 type IV collagen (together with some α3–α6 chains) and fibronectin, including cellular isoform (Fig. 2D), that were not observed normally on the endothelial face of the DM. Thus, PCL was an accumulation of corneal stromal and BM components. This ECM-type of PCL resembled the ECM-type of SEF. In two cases, well-developed PCLs with myofibroblasts inside them (Figs. 5D to 5F) were found (see also), apparently corresponding to the fibrocellular-type SEF.

DISCUSSION

PBK/ABK remains a leading indication for corneal transplantation, but there is little basic information
Extracellular Matrix of Bullous Keratopathy Corneas

Concerning this disorder, studies dispute the theory that intraocular lenses affect the protein levels or complement factor C3 or C5 in aqueous humor that could play a role in inflammation. The stroma of the PBK/ABK corneas has decreased keratan sulfate content, but the distribution of types V and VI collagen does not change. One study described lack of laminin, fibronectin, and type IV collagen in the epithelial BM of PBK/ABK corneas. PBK/ABK corneas often have a thickened DM with an accumulation of new fibrillar ECM with or without cells (PCL) between the endothelium and the DM. This ECM contains type I and V collagen, as well as some type III collagen, that are not present in this location in normal corneas.

Here, the evidence for ongoing fibrosis in PBK/ABK corneas is provided. In 16 of 20 cases, a fibrotic PCL containing stromal ECM and BM components was seen, and in 10 of 20 cases, SEF with ECM deposition was observed. It should be noted that the abnormal fibrotic deposition of ECM proteins was detected even in PBK/ABK corneas without evidence of SEF or PCL formation. The most conspicuous alteration of ECM pattern in PBK/ABK corneas was an induction of stromal tenascin.

Tenascin is a large hexameric glycoprotein composed of multiple domains. The tenascin family comprises three proteins that are different gene products: tenascin-C (the first discovered), tenascin-R (restrictin), and tenascin-X (X gene product). They may play a role in cell migration and proliferation, tissue repair, and oncogenesis. In addition, alternative splicing of fibronectin type III-like (FN-III) repeats generates a number of isoforms. Tenascin is antiadhesive. Cells may attach to it but do not spread well. The cell-binding sites are in the FN-III domains II–VI and in the fibrinogen-like domain, whereas the epidermal growth factor-like domain is antiadhesive. Tenascin can weaken cell-substrate attach-
FIGURE 5. Posterior collagenous layer in PBK/ABK corneas. (A to C) Extracellular matrix-type PCL containing types VIII (A) and XIV (B) collagen and entactin/nidogen (C). (D to F) Fibrocellular-type PCL containing types VI (D) and XII collagen (E) and numerous myofibroblasts positive for α-smooth muscle actin (F). S = stroma; P = posterior collagenous layer. Bar = 40 μm.

Tenascin may be upregulated in PBK/ABK by the following mechanism. In the diseased corneas, stromal fluid accumulation and a resultant swelling may create a significant mechanical stress to stromal keratocytes. In this situation, the cells could attempt to relieve the strain and preserve normal stromal structure by contracting their surrounding collagen. In cultured fibroblasts, such collagen contraction has been shown specifically to induce production and deposition of tenascin.48 It is thus possible that tenascin induction in PBK/ABK corneas could be triggered by mechanical stress. Some data seem to support this assumption. The degree of edema in PBK/ABK-failed corneal grafts is higher than in primary PBK/ABK.49 According to the stress hypothesis, the failed grafts should have more stromal tenascin than primary PBK/ABK corneas. Indeed, all four failed grafts studied here showed marked staining for tenascin in midstroma, in addition to anterior and posterior stroma. However, of 16 corneas with primary PBK/ABK, staining for tenascin in midstroma was noted only in two, and it was weaker than in failed grafts (Table 1).

Another salient feature of PBK/ABK corneas was an alteration of BM structure. In accordance with previous data,14 there was a reduction (up to a complete absence) of fibronectin, laminin-1, and entactin–nidogen in the epithelial BM. This could lead to a weaker epithelial adhesion to the Bowman’s layer and could facilitate bullae formation. The DM alterations in PBK/ABK corneas were also found. They primarily concerned α1–α2 type IV collagen and fibronectin. In normal-aged corneas, both proteins usually were seen on the stromal face of the DM.16 However, in PBK/ABK corneas, they were found on the endothelial face of the DM, irrespective of the presence or absence of a PCL (Figs. 2B, 2D; Table 2). It is unclear
which cells are responsible for these changes in PBK/ABK. They could be keratocytes, which are presumably the normal source of fibronectin and of α1-α2 type IV collagen in the DM. On the other hand, endothelial cells, which apparently do not deposit these proteins in normal corneas, could acquire an altered ECM pattern during PBK/ABK development. It is noteworthy that cultured corneal endothelium responded to the endogenous basic fibroblast growth factor by modulating ECM production from type IV to types I and V collagen. Because basic fibroblast growth factor can induce fibrosis, it might contribute to the changes of the DM composition in PBK/ABK.

A common feature of PBK/ABK corneas is the formation of PCL and of bullae with subsequent SEF. This article provides a detailed description of the ECM molecular composition in these areas. In both cases, abnormal ECM had a similar composition and was a mixture of stromal (tenascin, decorin, and collagen types I, III, V, VI, VIII, XII, and XIV) and BM components (type IV collagen, laminin-1, entactin-nidogen, perlecain, bamacan, and fibronectin). Importantly, PCL contained tenascin in all cases. This antiadhesive glycoprotein could provide an ECM environment for the endothelial cells in which they would be unable to attach normally, to spread, and to maintain normal corneal hydration (see also53). Studies are under way to determine which cells produce tenasin in the PBK/ABK corneas, which cytokines trigger its induction, and which tenasin splice variants are expressed.

Staining for stromal components was considerably stronger in PCL and in SEF than for most BM components (see, for example, Fig. 4), suggesting that cells that normally produce stromal ECM (apparently keratocytes) may contribute to its abnormal accumulation in PBK/ABK corneas. However, in the stroma of such corneas, the major ECM components did not seem to be affected. Possibly, the epithelial and endothelial cell phenotype in PBK/ABK could be modulated toward the production of an ECM (in ECM-type SEF and PCL) normally present in the stroma (see also50,51), with decreased deposition of BM components. This could be an attempt of the cells to increase their adhesion to the ECM in edematous conditions and preserve the corneal integrity. In fibrocellular-type SEF and PCL, myofibroblasts may be the source of abnormal ECM. In SEF, myofibroblasts seem to originate from stromal elements but not from epithelial cells or their limbal precursors because they do not contain epithelial markers (keratins). In fibrocellular-type PCL, the source of its fibroblast-like cells (myofibroblasts) may be modulated endothelium.

Following the primary defect in PBK/ABK corneas, possibly at the endothelial level, an active fibrosis ensues that definitely contributes to the disease progression. In fact, there is a correlation between the decrease in visual acuity of patients and the appearance of tenascin in midstroma that may be a marker of advanced fibrosis. The major ECM abnormalities found in PBK/ABK corneas cannot be explained easily by an altered fluid balance. Vision-threatening changes may result from abnormal production and deposition of ECM by dysfunctional cells, which may exacerbate the disease. By inference from other systems, excessive ECM production in PBK/ABK corneas could be modulated by fibrogenic cytokines, transforming growth factor-β1 (−β2), basic fibroblast growth factor, or platelet-derived growth factor. Directional modulation of the levels of involved cytokines may make it possible to normalize corneal ECM and to slow down or stop the disease progression, as demonstrated for fibrotic diseases in other organs. Only then, transplantation of normal endothelium to restore its function could become feasible.

**Key Words**

basement membrane, bullous keratopathy, corneal edema, extracellular matrix, immunocytochemistry

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