The synthesis of basement membrane by the corneal epithelium in bullous keratopathy

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With the aid of the electron microscope, the ultrastructural changes associated with bullous keratopathy were studied in corneal specimens from a patient with congenital hereditary corneal dystrophy and from one with congenital glaucoma. Within the subepithelial bullae of both specimens, infoldings of the basal plasma membrane of the basal epithelial cells could be observed to contain basement-membrane-like material accompanied by hemidesmosomes. Morphologically identical membranous infoldings and short segments of basement membrane were found along the basal epithelial surface of rabbit corneas in which basement membrane regeneration had been induced by superficial keratectomy. On the basis of these ultrastructural similarities, it is therefore suggested that the formation of subepithelial bullae with the concomitant interruption of the basement membrane induces the corneal epithelium to attempt restoration of the integrity of this layer by the synthesis and deposition of new basement membrane material.

Key words: congenital hereditary corneal dystrophy, congenital glaucoma, corneal stroma, corneal epithelium, Bowman's membrane, corneal basement membrane, keratectomy, ultrastructure.

While investigating the histological and ultrastructural pathology of the cornea in a case of congenital hereditary corneal dystrophy, several alterations of the epithelial basement membrane of that cornea were discovered which invariably occurred in conjunction with the presence of small subepithelial bullae. In particular: (1) at the edge of every bulla, the basement membrane and its attendant hemidesmosomes abruptly ceased; (2) inside the bullae, the basal-cell plasma mem-

brane appeared intact, and the underlying surface of Bowman's layer was covered only by amorphous debris without trace of basement membrane; and (3) infoldings of the basal plasma membrane of the basal cells within the bullae enveloped aggregates of material which closely resembled short segments of basement membrane. (Refer to Fig. 14 of reference 8 for illustration of these findings.)

Hypothesizing that these ultrastructural changes might not be unique to congenital hereditary corneal dystrophy (CHCD) but rather that they might represent the fine-structural picture of epithelial bullous changes in general, a corneal specimen with marked bullous keratopathy from a patient with congenital glaucoma was also studied. The purpose of this report, therefore, is: (1) to present in greater detail the basement membrane changes occur-

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Corneal epithelium synthesis of basement membrane

ring within the bullae of the CHCD specimen; (2) to compare the CHCD findings with the basement membrane morphology observable in the congenital glaucoma material; and (3) to discuss the possible functional significance of such basement membrane configurations.

Materials and methods

The following tissue was prepared for electron microscopy according to the previously described procedure: (1) the whole normal cornea from the enucleated eye of a patient with a malignant posterior melanoma, (2) the corneal button removed at keratoplasty of the patient with CHCD, (3) the entire cornea from the enucleation specimen of a patient with uncontrollable congenital glaucoma, and (4) whole rabbit corneas with regenerating epithelium and basement membrane from young adult albino rabbits of the New Zealand giant strain which were killed at various intervals after partial lamellar keratectomy. (These surgical procedures were performed by Dr. Ali A. Khodadoust using the technique previously described by him.)

All the electron micrographs utilized in this study represent material sectioned in a perpendicular plane with respect to the corneal surface, so that the misleading appearances and interpretative uncertainties of tangential sectioning might be avoided.

Results

Normal human cornea. For purposes of orientation and comparison, an electron micrograph (Fig. 1) is included to illustrate the typical ultrastructural appearance of the basement membrane of the normal human cornea. The major features of the epithelial-stromal junction (which is mediated by the basement membrane) may be seen in Fig. 1 to consist of: (1) a smooth, continuous plasma membrane that covers the basal surface of the basal epithelial cells (cf. the considerable interdigitation and infolding of the lateral plasma membranes of these cells); (2) numerous attachment specializations (hemidesmosomes) which are spaced along the basal plasma membrane; (3) the subjacent basement membrane, composed of an electron-dense band, which varies between 250 A and 500 A in thickness and which closely follows the contour of the basal plasma membrane, being separated from this plasma membrane by an electron translucent zone (the lamina lucida) of fairly constant thickness (250 to 400 A); and (4) the underlying feltwork of disorganized collagen fibrils, which constitutes Bowman's layer of the stroma.

Congenital hereditary corneal dystrophy. Fig. 2 shows at low magnification the central area of a subepithelial bulla (similar to the one illustrated by Fig. 14 of the previous report of this CHCD specimen). Even in this low-power survey electron micrograph, the absence of an intact basement membrane, the integrity of the basal plasma membrane (with its apparently basement-membrane-lined infoldings), and the debris-covered surface of Bowman's layer are quite evident. Fig. 3 and 4 are higher magnifications of areas along the basal epithelial surface shown in Fig. 2, while Fig. 5 depicts a corresponding area from a nearby bulla. In these electron micrographs, the highly convoluted basal-cell plasma membrane forms pockets which contain small focal aggregates of material with an amorphous quality and electron density that is highly suggestive of basement membrane. In addition, the basement-membrane-like substance: (1) consistently assumes a bandlike configuration, about 700 to 1,000 A in thickness, which is comparable to the form of the normal basement membrane, although somewhat thicker (cf. Fig. 1); and (2) is always separated from the encapsulating cell membrane by an electron-translucent space of about 700 A, not unlike the lamina lucida of the normal basement membrane. Still further evidence of the identity of this material is the presence of numerous hemidesmosomes along the basal cell membranes in only those loci where basement-membrane-like material is found.

In some cases (Fig. 5) the apparent isolation of these pockets from the cell surface is perhaps an indication of their existence as vesicles within the basal cell.
Figs. 1 and 2. For legends see opposite page.
cytoplasm. In other instances (Fig. 4), the membrane encircling the basement membrane deposits seems to be continuous with the basal cell membrane via only a narrow channel, whereas in still other areas this connection is entirely obvious (Figs. 3 and 5). And indeed, a few pockets appear to have become entirely everted, thereby exposing small discontinuous segments of basement membrane to the basal cell surface (Figs. 3 and 4).

Finally, the high-magnification view of Bowman's layer (Fig. 6) in an area similar to that of Fig. 2 reveals that only rarefied amorphous material, quite unlike basement membrane, covers the denuded surface of this collagenous layer.

**Congenital glaucoma.** Figs. 7 through 11 illustrate various areas along the epithelial-stromal junction of the congenital glaucoma cornea. A relatively normal zone is depicted in Fig. 7. Here the epithelial cells are closely apposed to Bowman's layer and are attached to the intervening basement membrane by several hemidesmosomes. The basement membrane is, however, nonuniform and extremely attenuated over some intervals, although in others somewhat thickened (700 to 900 Å). As was similarly noted in the CHCD specimen, the occurrence of hemidesmosomes is seen to closely parallel the presence of basement membrane. As the edge of a subepithelia bulla is approached, the basement membrane invariably ends quite abruptly (as in Fig. 8) and hemidesmosomes are no longer found along the basal-cell plasma membrane, which now lies directly on the surface of Bowman's layer.

Within a bulla, the picture is again remarkably similar to that of CHCD; as illustrated in Figs. 9 and 10, infoldings of the basal-cell plasma membrane are lined with basement-membrane material and possess hemidesmosomes only where such basement membrane is present. Moreover, within the bulla there are no remnants whatsoever of basement membrane along the surface of Bowman's layer which has become separated from the epithelium. And finally, Fig. 11 (taken from a similar location) constitutes the most convincing evidence that basement-membrane-containing pockets found at the basal cell surface can, in fact, also exist deep within the basal cell cytoplasm (up to 4 µ in Fig. 11), perhaps as discrete membrane-limited vesicles.

**Discussion**

From the remarkable similarity of the basement membrane configurations found along the basal margin of the basal cells within the subepithelial bullae of both CHCD and congenital glaucoma corneas, it is clear that basement-membrane-containing infoldings of the basal-cell plasma...
Figs. 3 to 5. For legend see opposite page.
Corneal epithelium synthesis of basement membrane

membrane are not unique to CHCD. Instead they must undoubtedly represent a part of the generalized electron-microscopic appearance of bullous keratopathy. Having established this common ground, it remains to determine the functional significance of these basement membrane aggregates. Given the absence of normal basement membrane in the bullous areas, there are two alternatives: (1) the infoldings (and vesicles?) represent the means for pinocytotic ingestion of the original basement membrane by the cells prior to its eventual intracellular destruction (implying that the membrane must have remained adherent to the basal epithelial cells as the accumulation of fluid forced the separation of the epithelium from Bowman’s layer), or (2) the infoldings (and vesicles?) are a part of the mechanism of basement membrane synthesis, being formed within the cell and eventually uniting with the basal plasma membrane to produce a new basement membrane immediately subjacent to the basal cells.

In an attempt to determine the correct alternative, partial lamellar keratectomies were performed upon normal laboratory rabbits. The obvious value of this type of preparation in relation to the present question is that in performing a keratectomy, both the epithelium and its basement membrane (as well as the superficial stromal fibrils) are removed, so that any basement membrane found thereafter within the keratectomy site must have been produced anew by the regenerated epithelial cells. In these preparations, the restoration of the epithelium and its basement membrane over the denuded stromal area was allowed to progress for varying lengths of time before enucleation of the eye and fixation of the cornea for electron microscopy. The resultant evidence from these experiments and from the literature served to rule out the first alternative while strongly supporting the second, as follows.

1. In the rat cornea, Jakus4 found that the epithelial basement membrane adhered strongly to both the stroma and the epithelium, for when the epithelium was torn away from the stroma, the tear was always just anterior to the cell membranes of the basal cells (that is, within the basal cell cytoplasm). The same situation prevails in keratectomized rabbit corneas when sufficient recovery time has allowed adequate renewal of the basement membrane; if the regenerated epithelium is pulled away from the stroma, the basal cells rupture, leaving segments of basal-cell plasma membrane and newly synthesized basement membrane attached together on the stromal surface.7 Moreover, Stehbens,14 using rabbit aortas perfused with Tyrode’s solution in order to induce bullous formation between the endothelium and the underlying connective tissue, has shown that the endothelial basement membrane tends to remain attached to the connective tissue, so that in areas where hemidesmosomes are few, the basement membrane separates completely from the bulla-elevated endothelial cells.

Figs. 3-5. Figs. 3 and 4 reproduce at higher magnification areas along the basal epithelial surface within the bulla shown in Fig. 2, while Fig. 5 shows a similar area from a nearby bulla, also from the CHCD cornea. In these electron micrographs, infoldings of the basal-cell plasma membrane (BCM) contain small focal aggregates of basement-membrane–like material (lettered). In some cases, (A) these infoldings appear to be isolated from the basal cell surface. In other instances, (B) only a narrow connecting channel (particularly at *) is evidence of continuity between the infolded membrane and the basal plasma membrane, whereas in other areas (C), this connection is entirely obvious. Also, small discontinuous segments of basement membrane (D) are frequently found entirely exposed to the basal cell surface. Note that in all situations the presence of hemidesmosomes (HD) closely parallels the occurrence of basement membrane material. (Fig. 3, ×35,000; Fig. 4, ×30,000; Fig. 5, ×35,000.)
Similarly, in the formation of corneal subepithelial bullae, therefore, it may be presumed that the basement membrane does not adhere to the underside of the basal cells but instead remains attached to the stromal surface. Thus, the basement-membrane-containing pockets are unlikely to be the result of pinocytotic uptake of old basement membrane.

2. The intracellular origin of epithelial basement membranes has been convincingly demonstrated by combined immunologic and electron-microscopic studies. Indeed, these investigations indicate that intracytoplasmic basement membrane synthesis (within the rough-surfaced endoplasmic reticulum) occurs in epithelial cells actively forming basement membrane, but that very little synthesis of basement membrane can be demonstrated in adult epithelium. On these grounds, the ultrastructural basis for basement membrane formation should be readily distinguishable in epithelial cells where a demand for new basement membrane exists, and should not be demonstrable in non-synthesizing, steady-state epithelium. Indeed, infoldings of the basal-cell plasma membrane containing short lengths of basement membrane are never apparent within normal stationary corneal epithelium. Thus, these particular specialized configurations of the basal cell surface presumably must represent some aspect of the epithelial cells' synthetic response to the need for new basement membrane.

3. In the superficially keratectomized rabbit cornea, a recovery period of 4 days is sufficient to accomplish preliminary re-epithelization over a keratectomy area of 5 mm. diameter. Complete restoration of the basement membrane of this epithelium is not accomplished, however, for 6 weeks or more. During this interval, the progressive synthesis of new basement membrane could be monitored in these corneas as is depicted in Figs. 12 and 13, which show areas along the interface between the basal epithelial cells and the stroma near the center of the lesion after only 7 days of postoperative regeneration. Here basement-membrane-lined infoldings of the basal-cell plasma membrane, are especially prominent (as in Fig. 12) where the basal cell surface is quite flat and the membranous connecting channels are particularly narrow. A similar situation is illustrated in Fig. 13 where short, relatively linear segments of basement membrane and their attendant hemidesmosomes are found exposed to the stromal collagen, as if by simple eversion of the contents of single membranous pockets. Moreover, the independence of the epithelium in pro-
Figs. 7 & 8. Electron micrographs of the epithelial-stromal junction of the congenital glaucoma cornea. In Fig. 7, a relatively normal area is shown in which the basal epithelial cells (B Ep) are closely apposed to Bowman's layer of the stroma (Str) and attached to the intervening basement membrane (BM) by several hemidesmosomes (HD). This basement membrane is, however, nonuniform and extremely attenuated over some intervals, although somewhat thickened in others. (In addition, a typical corneal nerve may incidentally be seen in cross-section as a nonmyelinated C-fiber, containing numerous small mitochondria and vesicles, and coursing between two basal cells.) In Fig. 8, as the edge of a bulla (B) is approached, the basement membrane and its attendant hemidesmosomes abruptly cease to exist so that the basal-cell plasma membrane (BCM) here lies directly on the surface of Bowman's layer. (Fig. 7 ×32,000; Fig. 8 ×42,000.)
Figs. 9 to 11. For legend see opposite page.
ducing these basement-membrane-lined infoldings is demonstrated in Fig. 14, taken from the central area of a subepithelial bulla where the loosely adherent epithelium had been lifted away from the keratectomy bed by fluid accumulation. In these preparations, even though similar basement membrane collections could not be found at sufficient depth within the basal cell cytoplasm to constitute definitive evidence of their presumed intracytoplasmic origin, the additional presence of small (0.2 μm diameter) perinuclear vesicles, each containing amorphous material suggestive of basement membrane, is entirely consistent with this concept. Thus, although the present evidence for the vesicular origin of these infoldings essentially rests upon Fig. 11 alone, it is clear that the occurrence of basement-membrane-lined plasma membrane infoldings and discontinuous basement-membrane segments at the basal surface of epithelia lacking normal basement membrane must, in fact, represent at least part of the mechanism of basement membrane renewal.  

Though the presence of these plasma membrane infoldings has not been previously reported, the foregoing interpretation is, however, indirectly supported by the observations of several other investigators. Jakus, studying the healing of perforating wounds in the rabbit cornea, found the epithelial basement membrane to be initially incomplete, with only short segments and associated hemidesmosomes present along the basal side of the epithelial layer. Thomas, investigating the synthesis of Schwann cell basement membrane during peripheral nerve regeneration, observed that new basement membrane first appeared in short lengths with free ends. Salpeter and Singer reported that in the initially healing limb bud of Triturus, following amputation, the epidermis is separated from the dermis by a basement membrane showing many gaps which eventually fill to give an intact basement membrane. All of these findings are consistent with the proposed synthesis of basement membrane as a series of discrete lengths which coalesce, in time, to produce a continuous subepithelial layer.  

The role of the basement membrane in securing the tight adhesion of epithelium to stroma, and the obvious importance of this close union, have been demonstrated many times. To cite only a few studies which are of particular relevance: (1) In the keractectomized rabbit cornea, the progression from loose to tight adhesion of regenerated epithelium follows the time course of basement membrane regeneration rather than re-epithelialization. If the epithelium is only scraped away, leaving an intact basement membrane which can be reutilized by the recovering epithelium, tight adhesion is accomplished almost as soon as the epithelium has regenerated. (2) Forgacs found that bulbar keratitis could be induced experimentally (using Cogan’s method) to a much greater degree if trypsin, which is known for its destructive effect on basement membrane, was applied to the cornea. (3) Goldman (personal communication) has discovered that segments of the basement membrane are missing in specimens from cases of recurrent corneal erosion, with the lack of adhesion between epithelium and stroma facilitating the

Figs. 9-11. Electron micrographs of the congenital glaucoma cornea in which subepithelial bullae have separated the basal epithelial cells from the stroma (Str). The intact basal-cell plasma membrane (BCM) exhibits extensive infoldings that are lined with basement membrane material (BM) and that possess hemidesmosomes (HD) only where such basement membrane is present. No remnants of basement membrane are visible along the surface of Bowman’s layer. In Fig. 11, basement-membrane-containing pockets are apparent up to 4 μ deep within the basal cell cytoplasm. (Fig. 9, x40,000; Fig. 10, x40,000; Fig. 11, x30,000.)
Figs. 12 to 14. For legend see opposite page.
production of recurrent epithelial defects. Similarly, Griffith and Fine have shown the presence of abrupt localized absences of both basement membrane and adjacent hemidesmosomes in the biopsy specimen of a superficial corneal dystrophy of probable early Reis-Bucklers' type in which corneal irritation and epithelial breakdown frequently occurred.

In summary, it would seem that the basal cells of the corneal epithelium naturally seek to maintain the integrity of the bond between epithelium, basement membrane, and stroma. Hence, in situations where the basement membrane is injured or otherwise removed, as in bullous keratopathy, the immediate response of the epithelial cells is the induction of basement membrane synthesis in an attempt to restore firm attachment once stromal contact is again established. It is our impression that basement membrane material and hemidesmosomes are manufactured concurrently along folded membranous surfaces, existing either free within the cell as vesicles or attached to the basal plasma membrane as infoldings. (Simultaneous production of basement membrane material and hemidesmosomes would then account for their invariably mutual presence or absence in the pathological conditions of CHCD, congenital glaucoma, recurrent corneal erosion, Reis-Bucklers' superficial corneal dystrophy and keratoconus as well as in normal frog lymphatic endothelium and in experimentally keratectomized corneas.) Given the synthesis of discrete lengths of basement membrane material in this manner, the subsequent deposition of these lengths, piece by piece, along the basal surface of the corneal epithelium might then accomplish the restoration of a continuous basement membrane layer.

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Figs. 12-14. Electron micrographs of the superficially keratectomized rabbit cornea after 7 post-operative days. In Fig. 12, the regeneration of basement membrane is evidenced by basement membrane-lined infoldings (BM) of the basal-cell plasma membrane (BCM). Similarly, in Fig. 13, short relatively linear segments of basement membrane are found at the basal surface of the basal epithelial cells (B Ep), directly exposed to the stromal collagen (Str). In Fig. 14, taken from the central area of a subepithelial bulla in this same specimen, basement membrane-lined infoldings of the basal plasma membrane are again apparent. Note that in all instances, hemidesmosomes (HD) are evident only in areas where basement membrane is also present. (×41,500.)


