but when the difference exceeds a certain level, the binocular system is unable to fuse these perceived image differences and may function as binocular inhibition resulting in a smaller binocular than monocular VER amplitude. Our results show that the binocular system may be able to compensate for a perceived retinal image size difference between two eyes of up to 3.0%. Although a direct comparison cannot be made, our results are in good accordance with those obtained from psychophysical and clinical studies. Whereas Lovasik and Bishop\(^6\) reported that simulated aniseikonia did not affect the amplitude and implicit time of transient pattern reversal VER, we found steady-state pattern reversal VER very sensitive to the perceived retinal image size differences between two eyes. An abnormal degree of binocular summation suggests defective binocular function. However, asthenopia and headache can be observed in patients with passable VER examination. Further clinical studies are required to clarify these findings. We suggest that pattern reversal VER may be helpful in evaluating visual function, especially binocular function of pediatric subjects in whom subjective methods are of limited use.

**Key words:** aniseikonia, binocular interaction, binocular summation, binocular vision, pattern reversal VER

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**Human Basement Membrane Components of Keratoconus and Normal Corneas**

John A. Millin, Barry M. Golub, and C. Stephen Foster

Using immunofluorescence techniques, we analyzed the distribution of glycoproteins in normal and keratoconus corneas of humans. Laminin, bullous pemphigoid antigen, fibronectin, and fibrin/fibrinogen were all found in the epithelial basement membrane of normal corneas. Keratoconus corneas produced similar results, except that staining for fibrin/fibrinogen was weak. Fibrin/fibrinogen was absent from normal corneal basement membrane in animal models studied previously. Keratoconus may be the result of the lysis of fibrin or may involve impeded elaboration of fibrin. Invest Ophthalmol Vis Sci 27:604-607, 1986

Normal corneal basal epithelial cells are anchored to the stromal substratum at the basement membrane zone (BMZ) through hemidesmosome attachment complexes. Endothelial cells are anchored by hemidesmosomes to their basement membrane, Descemet's membrane. These basement membranes are similar to epidermal basement membrane, including ultrastructurally identifiable electron dense (lamina densa) and electron lucent (lamina lucida) zones. Bullous pemphigoid antigen\(^1\) has been localized to the lamina lucida, while laminin,\(^2\) Type IV collagen,\(^3\) and fibronectin\(^4\) have been localized to the lamina densa.

Ultrastuctural derangements of these zones of the BMZ have been reported in keratoconus.\(^5\) Keratoconus, a central, noninflammatory, usually bilateral

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**References**

ectasia of the cornea, is of unknown etiology. Progressive thinning and scarring result in an irregular, cone-shaped protrusion of the central cornea.

We analyzed the distribution of glycoproteins in normal and in keratoconus corneas using immunofluorescent techniques. We then correlated our findings with known ultrastructural features of this disease.

**Materials and Methods.** Eight keratoconus corneas were obtained at the time of penetrating keratoplasties performed at the Massachusetts Eye and Ear Infirmary. Eight normal, age-matched corneas were obtained from the New England Eye Bank. All corneas were embedded fresh in OCT compound (Ames Company; Elkhart, IN) for cryostat sectioning.

Fluorescein (FITC)- and rhodamine (TRITC)-conjugated goat anti-human IgA, IgG, IgM, albumin, Ig(A, G, M), and fibrinogen were obtained commercially, as were fluorescein-conjugated goat anti-rabbit IgG, fluorescein-conjugated rabbit anti-sheep IgG, and unconjugated rabbit anti-human fibronectin (Cappel Laboratories; Cochranville, PA). Sheep anti-laminin was kindly provided by Dr. George Martin. Autoantibodies in serum from a patient with bullous pemphigoid were used to detect bullous pemphigoid antigen.

Cryostat sections (4 μm thick) were washed with PBS and incubated for 30 min in a moist chamber at room temperature with one of the following: fluoresceinated goat anti-human IgA (1:16); fluoresceinated goat anti-human IgG (1:30); fluoresceinated goat anti-human fibrin/fibrinogen (1:16); rhodamine-conjugated goat anti-human IgM (1:8); rabbit anti-human fibronectin (1:50) followed by fluoresceinated goat anti-rabbit IgG (1:40); and sheep anti-laminin (1:140) followed by fluoresceinated rabbit anti-sheep IgG (1:12). Sections to be studied for distribution of bullous pemphigoid antigen in the BMZ were first incubated with unconjugated goat anti-human IgG (1:30) to block all IgG antigenic sites normally found in corneal tissue. The tissue was then washed in PBS and incubated with human antiserum to bullous pemphigoid antigen (1:20) followed by fluoresceinated goat anti-human Ig(A, G, M) (1:400). As a control for all indirect immunofluorescent stains, PBS was used in place of the primary antiserum to verify that the secondary, fluoresceinated antiserum was not nonspecifically staining the tissue. Sections incubated with fluoresceinated goat antiserum to human albumin were used as controls for passive serum leakage. A Zeiss Photomicroscope III (Carl Zeiss, Inc.; Oberkochen, West Germany) equipped with epi-illumination and phase optics was used throughout the study. The intensity of staining was graded on a scale of 0 to 4+ by a masked observer.

**Results.** Normal BMZ stained intensely for fibrin/fibrinogen (the antibody does not distinguish fibrin from fibrinogen). We also found fibronectin (Fig. 1A), laminin, and bullous pemphigoid antigen in normal epithelial basement membrane. Descemet's membrane was positive for laminin and fibronectin. The epithelium was slightly positive for fibrin/fibrinogen. There was no epithelial staining with the other antisera. The stroma was diffusely positive for IgA, IgG, fibronectin, and albumin. Many prominent fibrin fibrils were also noted in the stroma of normal corneas. These results of normal corneas are summarized in Table 1.

The keratoconus corneas demonstrated a normal

![Fig. 1. (A) Normal human cornea stained for fibronectin. Note the BMZ positivity. The epithelial background staining is easily distinguished from the positive BMZ. (B) Keratoconus cornea stained for fibronectin. The overall staining pattern is the same as the normal cornea stained for fibronectin and demonstrates a positive BMZ.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933128/)

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* Endothelium was absent from most specimens.
† Bullous pemphigoid antigen.
staining pattern in response to all antisera tested except for fibrin/fibrinogen. An example of the normal staining pattern of a keratoconus BMZ with anti-fibronectin is shown in Figure 1B. Whereas normal corneas consistently stained intensely at the BMZ for fibrin/fibrinogen, the keratoconus corneas stained weakly with this antiserum. All normal corneas stained with the anti-fibrin/fibrinogen antiserum were graded 4+ at the BMZ (Fig. 2A), whereas keratoconus corneas received an average grade of 1.25 (range 0 to 2) (Fig. 2B). Adsorption studies demonstrated the specificity of this finding: When anti-fibrin/fibrinogen antiserum was incubated with human fibrinogen coupled to CNBr Sepharose 4B beads (Pharmacia; Piscataway, NJ), no staining of the BMZ occurred when this adsorbed preparation was subsequently used as a reagent in the immunofluorescence analysis.

Discussion. We demonstrated that laminin, bullous pemphigoid antigen, fibronectin, and fibrin are components of normal human corneal epithelial basement membrane. These results suggest that there are important differences between humans and some animal models. In rabbits, fibrin and fibronectin are absent from normal BMZ; these glycoproteins do appear on wounded corneal surfaces and subsequently disappear after providing a matrix over which epithelial cells can migrate to cover the wound. Kurkinen et al have shown that fibronectin is absent from chick corneal BMZ.

Using Type IV collagen as a specific basement membrane marker, Newsome et al have shown that basement membrane destruction is one aspect of keratoconus. These investigators also found that keratoconus organ cultures produced normal amounts of collagen suggesting that the pathogenesis of the disease may be related to increased collagenolytic activity, rather than abnormal collagen production.

Collagen is the most abundant component of the corneal stroma. Corneal transparency depends on proper packaging and arrangement of the collagen bundles. Koa et al have suggested that increased collagenase activity is responsible for the stromal weakening and thinning seen in keratoconus.

Plasmin, through the activation of plasminogen-activating factor, mediates the lysis of both fibrin and collagen. The decreased fibrin we observed in the epithelial BMZ of keratoconus as compared to normal corneas could reflect plasmin activation, with resultant fibrin degradation. Abnormalities in the regulation of
plasmin-mediated lysis of collagen and fibrin may lead to the stromal thinning and basement membrane destruction seen in this disease. Another possibility is that the decreased fibrin seen in the keratoconus BMZ reflects decreased elaboration or diminished binding of this protein to other BMZ components. Further studies are in progress to determine whether keratoconus corneas exhibit increased plasmin activity.

Key words: keratoconus, cornea, fibrin/fibrinogen, basement membrane, glycoprotein

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Growth Characteristics of Primate (Baboon) Corneal Endothelium In Vitro*

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Primate (baboon) corneal endothelial cells have been grown continuously in vitro for over 40 passages. Endothelial cells migrated from the explants within three to five days. Growth became confluent by 14 to 21 days; the cells in culture were polygonal in shape and formed a compact monolayer. In passages one to four, cells divided with a doubling time of 72 hours; this increased to 96 hours after the 16th passage. Even though the general morphological appearance was unchanged in passages greater than 10, the cells became irregular, exhibiting an enlarged and elongated profile. The addition of epidermal growth factor to the medium resulted in increased cell growth. To our knowledge, there are no reported attempts to grow primate (baboon) endothelium continuously in tissue culture. Herein, we describe the establishment of continuous cell cultures from the corneal endothelium of baboons. These cells, with their anatomical, biological, biochemical, and phylogenetic resemblance to humans, are very valuable as a model system for corneal endothelial cell research.

Materials and Methods. Tissue: Eyes were obtained from baboons (Papio cynocephalus anubis), whose age varied from 2.2 years to 14.9 years, within 24 hr of enucleation. The corneas were immediately excised under sterile conditions and used either for dissecting the endothelium for tissue culture or for the preparation of stromal carriers by de-endothelialization as described below.

Culture: Endothelium was established as primary culture in a 25 CM² tissue culture flask (Corning lab-