COMPENSATORY EPITHELIAL HYPERPLASIA IN HUMAN CORNEAL DISEASE*

BY *Ralph C. Eagle, Jr*, MD,
Edward C. Dillon, MD (BY INVITATION) AND *Peter R. Laibson*, MD

INTRODUCTION

SINCE IT WAS FIRST DESCRIBED BY TROKEL AND ASSOCIATES,¹ THE 193-NM excimer laser has generated great excitement because of its potential application in surgical procedures designed to alter the shape of the anterior corneal surface. The excimer laser has been used experimentally to correct a variety of refractive problems using both peripheral radial keratectomy^{2,3} and central optic zone ablative procedures.^{4,5} Additional therapeutic applications for anterior corneal scarring, superficial dystrophies, and recurrent erosions also are under investigation.^{6,7}

A recent clinical report has implied that compensatory hyperplasia of the corneal epithelium (CEH) may be a factor in refractive regression and variation in refractive results after excimer laser photoablative procedures.⁸ This conclusion is based in part on data from histopathologic studies that have shown thickening of the corneal epithelium after excimer laser photoablation in animal and human eyes.^{4,8-11}

CEH is also observed during routine histopathologic examination in a variety of common corneal diseases marked by stromal loss or ectasia. To better assess the frequency and histopathologic features of CEH in several human corneal diseases, we conducted a retrospective histopathologic review of a large series of keratoplasty specimens excised from patients with keratoconus, chronic herpes simplex virus (HSV) keratitis, and non-specific corneal scarring.

*From the Department of Pathology and the Cornea Service, Wills Eye Hospital, Thomas Jefferson University, Philadelphia.

MATERIALS AND METHODS

A list of all corneal buttons coded as keratoconus, chronic HSV keratitis and nonspecific corneal scarring in the computerized diagnostic retrieval system of the Ophthalmic Pathology Laboratory, Wills Eye Hospital, during the 3-year period between May 1987 and May 1990 was generated. Routine histologic slides and pathology reports from all cases were retrieved from laboratory archives and reviewed retrospectively. All corneas had been fixed in neutral-buffered formaldehyde and embedded in paraffin. Routinely 6 μm sections stained with hematoxylin and eosin and periodic acid-Schiff were reviewed.

To assess for the presence of epithelial hyperplasia, the thickness of corneal stroma was measured centrally and peripherally with a calibrated ocular micrometer inserted as a reticule in the eyepiece of a standard laboratory microscope. Conversion figures for the microscope's several objective lenses were available, permitting thickness measurements at various magnifications. In general, the measurements were performed at the highest magnification possible. In cases with irregular stromal thinning or epithelial thickening, the thinnest or thickest area was measured and recorded. The number of cell layers composing the epithelium in each case also was estimated.

Slides from 15 eyes enucleated for posterior-segment uveal melanoma that had no known or apparent corneal pathology or glaucoma were used as normal controls to evaluate the degree of tissue shrinkage caused by fixation and processing.

RESULTS

In control material the corneal epithelium was 5 to 6 cells in thickness and measured 30 to 40 μm centrally and 40 μm peripherally. The stroma of the control corneas measured 500 to 550 μm centrally and 600 to 650 μm peripherally.

As expected, the corneal stroma was markedly thinned in most of the cases of keratoconus. In 87.7% (114 of 130), the central stroma measured between 100 and 300 μm ; most were approximately 200 μm . Peripherally, the stroma of the corneas with keratoconus was marginally thicker. In half (65 of 130) of the cases, the peripheral stroma measured less than 400 μm .

In 20% (26 of 130) of the cases of keratoconus, the corneal epithelium at the apex of the cone measured 60 to 100 μm in thickness and was composed of 7 to 12 layers of cells. Mild to moderate compensatory hyperplasia of the peripheral epithelium also occurred frequently. Over-

all, compensatory hyperplasia involving either the central or peripheral corneal epithelium, or both was observed in more than half (85 of 130, or 65.4%) of the cases of keratoconus (Figs 1 through 4).

Among the 36 cases of chronic HSV keratitis examined, 7 (19.4%) had stroma that was normal in thickness and epithelium that was either normal in thickness or attenuated. The remaining 29 herpetic corneas were characterized by markedly irregular variation in stromal thickness. (Figs 5 through 9) Usually the stromal thickness varied from 200 to 450 μm . In 62% (18 of 29) of the cases with variable stroma thickness, the epithelium had undergone varying degrees of facet-like hyperplasia that "filled-in" the areas of anterior stromal loss. In selected cases, epithelial facets measuring 1 to 2 mm in length and at least 60 μm in thickness were noted. In the most remarkable case the hyperplastic epithelium measured more than 20 cells and 200 μm in thickness.

In 56% (14 of 25) of the buttons coded as nonspecific corneal scars, hyperplastic epithelium covered areas of irregular stromal thinning. In these cases the hyperplastic epithelium ranged between 100 and 200 μm in thickness. The thickened epithelial plaques in all three categories of species contained scattered rounded cells with pyknotic nuclei (Fig 9).

FIGURE 1

Keratoconus. Epithelium is thickened overlying zone of greatest stromal ectasia at left. Arrow denotes mild peripheral CEH at right (hematoxylin-eosin, original magnification, $\times 10$).

FIGURE 2

Keratoconus with severe stromal ectasia. Epithelium has undergone marked compensatory hyperplasia in ectatic zone (hematoxylin-eosin, original magnification, $\times 25$).

FIGURE 3

Keratoconus with severe stromal ectasia seen in Fig 2. Higher magnification shows that hyperplastic epithelium constitutes nearly 80% of corneal thickness in ectatic zone. Bowman's membrane is attenuated and focally interrupted (hematoxylin-eosin, original magnification, $\times 100$).

FIGURE 4

Keratoconus. Thick plaque of hyperplastic epithelium overlying scarred and ectatic central stroma. Bowman's membrane persists peripherally (hematoxylin-eosin, original magnification, $\times 50$).

FIGURE 5

Chronic HSV keratitis with severe focal stromal loss. Tapering plaque of hyperplastic epithelium largely fills irregular defect in anterior stroma and roughly approximates smooth anterior corneal curvature (hematoxylin-eosin, original magnification, $\times 25$).

FIGURE 6

Chronic HSV keratitis with severe focal stromal loss. Thickened epithelial plaque filling defect in anterior stroma resembles large facet. Bowman's membrane is absent in ulcerated area (hematoxylin-eosin, original magnification, $\times 100$).

FIGURE 7

Chronic HSV keratitis with severe focal stromal loss. Thickened epithelium roughly approximates smooth anterior corneal curvature in area of deep stromal loss. Bowman's membrane is seen beneath CEH. Chronic inflammation persists in the anterior stroma (hematoxylin-eosin, original magnification, $\times 50$).

FIGURE 8

Chronic HSV keratitis with severe focal stromal loss. Bowman's membrane has been destroyed in this case. Stroma is chronically inflamed and vascularized. Epithelium at left is approximately five times normal thickness (hematoxylin-eosin, original magnification, $\times 100$).

FIGURE 9

Compensatory epithelial hyperplasia. Arrows mark nonviable epithelial cells with pyknotic nuclei in thickened epithelial plaque (hematoxylin-eosin, original magnification, $\times 250$).

These cells were thought to reflect the intraepithelial necrosis of single cells rather than dyskeratosis.

DISCUSSION

This retrospective histopathologic review of corneal epithelial morphology in corneas excised for keratoconus, chronic herpes simplex keratitis, and nonspecific corneal scarring indicates that CEH occurs frequently in several corneal diseases that often are marked by stromal ectasia or loss. Approximately half of the corneas in our series that had either nonspecific scarring or chronic herpetic keratitis showed epithelial thickening. In these cases the epithelium measured up to three to four times normal thickness and frequently formed facets that often extended 1 to 2 mm across the corneal surface. In 25% of the corneas with keratoconus, the epithelium was thicker than normal overlying the apex of the cone, corresponding to the area of greatest stromal ectasia. In addition, nearly one third of the keratoconus specimens showed mild epithelial thickening in the order of 60 to 90 μm in the periphery of the specimen. In most of these specimens Bowman's membrane was intact.

We believe that the mechanisms which normally are responsible for the maintenance of the corneal epithelium are operative in the pathogenesis of CEH. Thoft and Friend¹² have suggested that corneal epithelial maintenance depends on a balance between basal cell proliferation, the centripetal migration of cells from the limbus and the exfoliation of older surface cells. Eyelid closure may be a major driving force in epithelial cell migration and turnover.^{13,14} According to Lemp and Mathers,¹³ shearing forces generated by upper eyelid closure causes exfoliation of older superficial epithelial cells. This mechanism is somewhat analogous to a palpebral "snowplow." Lemp¹⁴ also indicates that the exfoliation of superficial epithelial cells appears to be decreased in depressed areas of the cornea where the cells are relatively protected from the shearing forces of the lid. Accordingly, the relative protection against eyelid-induced cellular exfoliation afforded by irregular areas of anterior stromal ectasia and loss may be an extremely important factor in the genesis of CEH. We infer that corneal epithelial cells that have migrated into depressed areas in the stroma continue to proliferate until the depressions are filled by epithelial plugs or facets. Exfoliation of the superficial cells (the major factor that determines epithelial thickness) commences when the anterior surface of the epithelial facet roughly approximates the level of the neighboring normal corneal surface and consequently becomes subject to the shearing forces of upper eyelid closure.

Thickening of the corneal epithelium has been shown histopathologically in both human and animal eyes after excimer laser photoablation.^{4,8-11} The results of our study imply that this cellular hyperplasia is a normal response of the corneal epithelium to stromal loss and is not intrinsically related to excimer laser therapy. We found that the degree of CEH appears to roughly correlate with the extent of corneal stromal loss. Extensive epithelial plaques generally occurred in corneas that had focal stromal loss. However, 65% of our cases of keratoconus, a disease which tends to have a more diffuse pattern of stromal loss, also had mild to moderate CEH. The presence or absence of Bowman's membrane did not appear to have an effect on the development of CEH. Our data complement previous reports that imply that compensatory hyperplasia of the corneal epithelium contributes to refractive regression and variation in refractive results after 193-nm excimer laser photorefractive keratectomy.

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DISCUSSION

DR JAY H. KRACHMER. When there is a loss of anterior corneal tissue, a natural healing process takes place in which the area is filled in by thickened epithelium. This is important because by far the largest percentage of refraction is that which occurs at the anterior cornea, or, more properly, the air-tear interface over the anterior cornea. What is a fortunate healing process in disease can become an unfortunate complication when anterior stromal tissue is purposely removed in an attempt to change the refractive power of cornea. In the case of excimer laser ablation for myopia, the refractive state of the eye moves from preoperative myopia, to postoperative reduction of myopia, to gradual movement back in the myopic direction.

Because of great interest in, and possible future approval of, medical devices for performing refractive surgery, the success of which depends significantly on the healing process, it is important to learn all we can about compensatory epithelial thickening. The authors undertook this study for that purpose.

The variables that make it risky to translate information about epithelial thickening in disease states such as keratoconus, herpes simplex keratitis, and non-specific corneal scarring to healing of the anterior cornea following excimer ablation are obvious. How do factors such as infection, inflammation, medications, greater irregularity, the neurotrophic component, and wearing of contact lenses work together and independently to effect epithelial healing? Do keratoplasty specimens from patients wearing contact lenses have more or less epithelial thickening compared with those who did not wear a contact lens?

I look at the present work not as a study that truly gives a frequency of compensatory thickening of the epithelium, but as one that demonstrates the enormous and variable potential of the epithelium to thicken. In some cases the epithelium was more than one half of the total corneal thickness.

The authors observed that the development of epithelial thickening occurred whether or not Bowman's membrane was present. In the case of excimer laser surgery, Bowman's membrane is excised.

The earlier work with the excimer laser resulted in significant regression of effect because of both epithelial thickening and stromal repair with thickening. To my knowledge, there have been no studies showing histologically that using a more homogeneous excimer beam and creating a smoother transition zone between the ablated cornea and the peripheral nonexcised cornea results in less regression. This is, however, the feeling of investigators with whom I have spoken. Perhaps the authors are aware of histologic confirmation for this clinical impression.

I feel that the authors of the present study on epithelial thickening are to be congratulated for furnishing us with more information on this very timely topic.

DR WALTER J. STARK. That is a very nice demonstration of the way the cornea wants to heal itself. That cannot be so beneficial in excimer laser for photorefractive keratectomy where one is trying to cure myopia or reduce the myopia. But that nice smooth layer of epithelial thickness has been very helpful for us in

phototherapeutic keratectomy where we have used the excimer laser to irradiate scars in the anterior one-third of the stroma. Initially, in our phototherapeutic trials we would debride the corneal epithelium before removing the scar. But the underlying surface was often very rough and irregular and that irregular surface could be just mapped down as we removed the scar, leaving the irregular surface. We tried various viscous agents to fill in the irregularity in the therapeutic keratectomies and then after thinking about it and going to Doctor Green and getting slides very similar to this of how the epithelium just wants to smooth that surface, we began leaving the epithelium on and treating through it to eradicate scars in the superficial one third of the cornea. I think our results have been better. In the discussion following Doctor Lindstrom's paper I will show histopathology after excimer laser treatment demonstrating essentially the same thing you all very nicely have shown in your paper.

DR RICHARD L. LINDSTROM. Thank you. I find this fascinating as well. We have postulated that when utilizing small optical zones in our excimer laser surgery the primary cause of regression is epithelial hyperplasia. There is some histology now being reported that confirms in several specimens that this epithelial hyperplasia does occur in humans. It can be associated with refractive regression. Primarily, these were therapeutic cases where we actually induced hyperopia inadvertently. I had one patient with herpes simplex whose refraction initially regressed back to about +7 to 8 and a postkeratoplasty specimen does show this compensatory epithelial hyperplasia, if you will. I have a question though, perhaps you can answer this and perhaps you cannot. First, why is the healing response primarily epithelial in the cornea? If you get a wound in your arm, for example, you get granulation tissue that fills in with connective tissue and collagen and the epithelial surface is relatively normal. Whereas in the cornea, we seem to get minimal stromal healing, much less than we initially expected in our excimer laser studies, but we are getting much more epithelial hyperplasia than we expected. Why does the cornea react differently than the rest of the body? Second, how does the cornea know when to stop remodeling the epithelium? What point does it go to and what might you theorize are the signals for compensatory epithelial hyperplasia to stop? If there is a point when it stops? Is there a way we might utilize this, for example, in excimer laser photorefractive keratectomy to our benefit. Thank you.

DR W. RICHARD GREEN. Doctor Eagle, I considered compensation epithelial thickening to be a normal healing response to maintain a smooth external surface. We observed this response within 24 hours after excimer laser ablation. The epithelial thickening is present at the margin of the ablated area within 24 hours. How can the epithelial thickening account for regression which occurs later on?

In radial keratotomies up to 38% of wound depth is healed by epithelial proliferation. With time the epithelial invagination is replaced by scarring and may be rotated to regression of the flattening effect. This is opposite epithelial thickening after excimer laser ablation. Maybe you can address these points.

DR RALPH C. EAGLE, JR. First, I would like to thank Doctor Krachmer for discussing our paper. In regard to his comments, I agree that there are many interesting factors that may be involved in compensatory hyperplasia of the corneal epithelium. I find Doctor Krachmer's comment about contact lens use particularly intriguing. Unfortunately, it was impossible to address all these factors in a retrospective histopathologic review. In regard to the occurrence of CEH in other corneal conditions, I did examine several cases of macular corneal dystrophy that were on file in my laboratory. In macular corneal dystrophy, the corneal stroma usually is thinner than normal, presumably because an inherited defect in the sulfation of keratin sulfate molecules affects corneal stromal hydration. I found no epithelial hyperplasia in the cases of macular dystrophy. I think that the regularity of the stromal loss in this condition may be an important factor. We generally observed massive thickening in corneas that had irregular stromal loss.

In regard to Doctor Lindstrom's question, it is recognized that the cellular factors involved in the healing of corneal and scleral wounds differ. Granulation tissue participates in the healing of scleral and limbal wounds. I think that the primary factor that distinguishes strictly corneal wounds is the avascularity of the cornea. You can't have granulation tissue without an ingrowth of blood vessels.

Doctor Lindstrom asked a final question about the nature of the signals that tell the epithelium to stop proliferating. I am quite certain that there is a lot of biochemistry going on here. Chemical mediators undoubtedly are involved. Our essentially mechanistic explanation was inspired by Lemp and Mather's work on corneal epithelial cell turnover. I speculate that epithelial cells in a depressed area of the cornea proliferate until they reach the plane of the neighboring normal stroma. When they reach this level they become subject to the shearing forces of upper eyelid closure and are "lopped-off" by the motion of the lid.