In vitro studies of corneal wound healing: epithelial-endothelial interactions

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Full-thickness explants of rabbit cornea were grown in organ culture and the migratory behavior of both epithelium and endothelium was observed under different experimental conditions. Following explantation, epithelial migration along the cut surface of the stroma began within six hours. By 24 hours, the epithelium reached the cut edge of Descemet's membrane and made contact with the endothelium at this point. Further epithelial advancement was then completely inhibited for up to 30 days following contact with the endothelium. Likewise, there was no migratory endothelial movement. If the endothelium was mechanically removed from the posterior corneal surface just prior to culture, the epithelium subsequently moved onto Descemet's membrane and completely encircled the explant after only 48 hours in vitro. Prior removal of epithelium permitted the endothelium to behave in a similar manner. However, endothelial migration was not completed until 96 hours in vitro because of a 48-hour delay before movement began. The results show that epithelium and endothelium mutually inhibit each other's forward movement and suggest an important role for this inhibitory interaction during corneal wound healing.

Key words: organ culture, corneal epithelium, corneal endothelium, contact inhibition, corneal healing.

The repair of perforating rabbit corneal wounds has been well described in morphologic studies and a dynamic sequence of events has been reconstructed from the morphologic data. However, little is known of the nature of regulatory controls governing behavior of the cell systems participating in the healing process. One such system is the corneal epithelium which shows inward migration along the cut edge of the stroma during the early phases of healing, followed by regression as the stromal tissue is repaired. In the human, failure of the epithelium to regress, as sometimes occurs following surgical and nonsurgical trauma to the cornea, may result in deep epithelial migration along the inner corneal surface and anterior chamber angle leading to obstruction of the aqueous outflow tract.
The study of control of epithelial migration is hindered by the existence of poorly defined noncorneal factors (e.g., tear film, limbal tissue, limbal vasculature, aqueous as well as clot formation, white blood cells, etc.) that influence wound healing. Thus, a tissue culture system may provide a more controlled environment for experimental analysis of epithelial behavior. Organ culture of the cornea represents a wound situation in that both epithelium and endothelium are confronted with a free edge as the result of the procedure of cutting the cornea into small full-thickness explants. Thus, the stage can be set for observations on cell movement in the absence of other concomitants of the healing process. The present in vitro studies show that in the rabbit cornea inhibitory interactions between epithelium and endothelium are important in limiting cell movement. The results suggest possible explanations for failure to control epithelial invasion seen occasionally following trauma to the human cornea.

Materials and methods

One-year-old-belted Dutch female rabbits were killed in a CO₂ chamber. The eyes were enucleated immediately and rinsed in sterile modified Eagle's minimal essential medium containing 10 per cent fetal calf serum and 100 units each of penicillin, streptomycin, and mycostatin (MEM-plus). The corneas were removed by sharp dissection and rinsed a second time in MEM-plus.

Corneal explants, 2 mm. in diameter, were then "punched out" with a trephine blade. The specimens were placed free-floating in plastic Petri dishes containing 2 ml. of MEM-plus and incubated at 37° C. in an atmosphere of 5 per cent CO₂ in air. The culture fluid was changed every three days. The dishes were agitated daily to prevent explants from attaching to the bottom surface of the dish. Explants which did become attached were dissociated with a sterile hypodermic needle. At appropriate intervals, specimens were fixed in a glutaraldehyde-formaldehyde mixture, and paraffin embedded by hand to minimize trauma to the tissue. Specimens were sectioned at 8 µ and stained with hematoxylin-eosin or periodic acid-Schiff.

In some experiments, the endothelium was removed by vigorously rubbing the posterior surface of the cornea with sterile cotton-tipped applicators prior to explantation in vitro. The efficacy of this method in completely removing the endothelium was confirmed by light microscopy. The endothelium-deficient explants were then cultured in the same manner as explants in which the endothelium was undisturbed. In other preparations the epithelium and a small portion of anterior stroma were removed by sharp dissection prior to incubation leaving the endothelium intact.

Results

In full-thickness explants, just prior to culture, both epithelium (Fig. 1, A) and endothelium (Fig. 1, B) were intact and extended to the cut edge. Occasionally, a few endothelial cells nearest the cut edge were inadvertently lost during the trauma of the trephining procedure. The cut surface of the stroma appeared relatively smooth. Following immersion in cul-
ture fluid, the corneal stroma began to swell and within 24 hours attained a maximal thickness of three to four times normal. Concurrent with the swelling the cut edge of Descemet's membrane curled inward toward the stroma generally assuming a triangular "cocked hat" appearance. At 24 hours, the leading edge of corneal epithelium had migrated over approximately 30 to 50 per cent of the lateral cut surface of the swollen stroma (Fig. 2). By 48 hours, the epithelium had arrived in the area of the cut edge of Descemet's membrane (Fig. 3). At this point, subsequent epithelial behavior was determined by the presence or absence of the endothelium. In the presence of endothelium, which itself showed minimal migratory activity by 48 hours, the epithelium generally failed to show any further forward movement over Descemet's membrane. After six days in culture, in 14 out of 14 explants, the epithelium had not advanced past its point of contact with endothelium relative to its position to Descemet's membrane (Fig. 4). At the point of contact, epithelial cells appeared to pile up to a depth of four to five layers in contrast with the one- or two-layered state seen during the early stages of migration. During the remainder of the 30-day observation period in an additional group of cultures, no further movement of the epithelium was observed relative to its position with respect to Descemet's membrane. By contrast, in the absence of endothelium, the epithelium advanced unimpeded over Descemet's membrane to completely encircle the explant within 48 hours (Fig. 5). After six days in culture, 13 out of 13 endothelium-free explants showed the typical multilayered epithelial cell epithelium over the entire extent of Descemet's membrane.

Additionally, in some preparations, the epithelium and a portion of anterior stroma were removed prior to culture, leaving the
Fig. 5. Light micrograph of a 48-hour explant from which endothelium was removed prior to being placed in vitro. Note that multilayered epithelium has moved onto Descemet's membrane (arrow). In this preparation, epithelium actually covered the entire extent of Descemet's membrane. x200.

Fig. 6. Light micrograph of 96-hour explant from which epithelium and anterior stroma were removed prior to being placed in vitro. Endothelium has migrated to completely surround the remaining stroma. x80.

endothelium intact. In this case the endothelium was observed to migrate off Descemet's membrane onto the lateral cut surface of the stroma following a lag period of 48 to 72 hours. By 96 hours, the endothelial monolayer completely surrounded the corneal stroma (Fig. 6).

In the stroma itself, by 24 hours the keratocyte nuclei had disappeared in a zone approximately 40 μm adjacent to the cut surface. Three to five days later, keratocyte nuclei were again found in this region but were not as plentiful as in other areas. No focal accumulation of keratocyte nuclei was noted in any area of the explant. By 9 to 14 days, keratocyte nuclei had disappeared from the posterior half of the stroma. By 30 days, keratocyte nuclei were present only in the upper quarter of the stroma.

Discussion

The normal healing of corneal wounds in the rabbit, as described by Matsuda and Smelser,1 involves an orderly series of cellular events including epithelial migration and regression in the anterior portion of the wound, endothelial migration and reformation of Descemet's membrane in the posterior portion of the wound, and keratocyte migration and ultimate collagen formation centrally. Under normal circumstances, epithelium and endothelium do not seem to make contact during wound healing. Abnormal wound healing in the human, manifested by uncontrolled migration of corneal epithelium into the anterior chamber, often results in partial or total loss of vision due to occlusion of the aqueous outflow tract and subsequent increased intraocular tension.2 The purpose of this study using rabbit cornea as a model system was to investigate patterns of epithelial and endothelial migration and possible regulatory interactions of the two cell types in vitro.

The results show that the onset of epithelial migration with a lag period of less than 24 hours and the onset of endothelial migration with a lag period of greater than 48 hours agrees substantially with in vivo observations in the rabbit.1 The more rapidly migrating epithelium, in the absence of endothelium, will completely encircle a 2 mm, explant within 48 hours. The endothelium, on the other hand, requires 96 hours to encircle an explant. In full-thickness preparations, due to the difference in rate of mobilization, the two cell types meet in the region of the cut edge of Descemet's membrane. No further movement of either cell type was observed relative to this point of contact, and there was no light microscopic evidence of extension of either cell type over or under one another. Thus, the cell types appear
to be mutually limiting further migration once cell-cell contact has been established.

The phenomenon of limitation of movement of cells following contact with other cells has been called contact inhibition of movement and was originally observed in vitro by Abercrombie and Heaysman and in vivo by Lash. The mechanism of contact inhibition of movement is unknown, although many theories have been proposed. It should be noted, however, that the expression of this phenomenon is not absolute and may be readily modified by environmental factors. For example, using chick fibroblasts Carter showed that contact inhibition of movement in vitro could be modified by changing the properties of the substratum to which the cells were attached. Under some conditions, the cells were inhibited and grew as a monolayer, whereas, under other conditions, their forward movement continued following collision with another cell so that one cell moved over the other leading to piling up. Contact inhibition of epithelial movement has not yet been demonstrated conclusively for the human cornea as it has for the rabbit. One could speculate, however, that abnormalities in epithelial behavior during human corneal wound healing might be explained on the basis of failure of this phenomenon to occur. In the case of traumatic laceration, for example, epithelial invasion of the anterior chamber might occur if the endothelium were sufficiently damaged or destroyed. In addition, even in the presence of an adequate endothelium, abnormalities in the environment (e.g., intraposition of herniated intraocular contents such as iris or vitreous, infection, altered blood flow, changes in pH, etc.) might sufficiently modify events at the point of epithelial-endothelial contact as to permit continued epithelial advancement. Alterations of pH in vitro have recently been shown to greatly modify the expression of contact inhibition. While the present study does not definitely define the problem of epithelial invasion of the eye in humans, it suggests possible future pathways of investigation.

REFERENCES