Human cornea organ cultures: epithelial-endothelial interactions

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Full-thickness explants of five human corneas grown in organ culture demonstrate that the migration of epithelium and endothelium is mutually limited once contact between the two cell types has been established. Two human corneas, however, failed to show this contact inhibition, and corneal epithelium surrounded completely the explant; both corneas showed cornea guttata. The implications of corneal epithelium-endothelium contact inhibition, and the failure of such inhibition, are discussed.

Key words: human cornea, organ cultures, contact inhibition, epithelial downgrowth.

The present study extends the work of Cameron and associates on corneal organ cultures in rabbits and that of Glickstein and associates on corneal organ cultures in dogs to corneal organ cultures in man. The previous work showed that the epithelium covered completely within 48 hours the cut edge of rabbit and dog corneal organ cultures and then made contact with the endothelium. After corneal epithelial-endothelial contact had been made, no further migration of either cell line occurred.

Organ culture of the cornea represents a wound situation in that both epithelium and endothelium are confronted with a free edge as a 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 studies in the human show that cornea inhibiting interactions between epithelium and endothelium are important in limiting cell movement. The results support previous explanations for failure to control epithelial invasion seen occasionally following trauma to the human cornea.

Material and methods
Seven corneal buttons were removed immediately following surgical enucleation. Six of the eyes were enucleated because they contained a malignant melanoma (patients' ages were 62, 67, 68, 72, 75, and 78 years) and the seventh eye, from a 13-year-old, was enucleated because of complications following cataract surgery.

The enucleated eyes were rinsed in sterile modified Eagle's minimal essential medium containing 10 percent fetal calf serum and 100 units each of penicillin, streptomycin, and myco-
statin (MEM-plus). The corneas were removed by sharp dissection and rinsed a second time in MEM-plus. Corneal explants, 3 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 percent CO₂ in air. The culture fluid was changed every 3 days. The dishes were agitated daily to prevent explants from attaching to the bottom surface of the dish. Explants which did become attached were dislodged 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, with the endothelium left intact.

Results

In full-thickness explants, just prior to culture both epithelium and endothelium 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 culture fluid, the cornea 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 percent of the lateral cut surface of the swollen stroma. By 48 hours, the epithelium had arrived in the area of the cut edge of Descemet's membrane. 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 (Fig. 1). After 6 days in culture, in five out of seven explants the epithelium had not advanced past its point of contact with endothelium relative to its position to Descemet's membrane (Fig. 2). 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 21 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.

Additionally, in some preparations the epithelium and a portion of anterior stroma were removed prior to culture, leaving the endothelium intact. In this case the endothelium was observed to migrate off Descemet's membrane onto the lateral cut surface of the stroma after a lag period of 48 to 72 hours. By 96 hours, the endothelial monolayer completely surrounded the corneal stroma.

In the stroma itself, by 24 hours the keratocyte nuclei had disappeared in a zone of approximately 40 μ adjacent to the cut surface. Three to 5 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 21 days, keratocyte nuclei were present only in the upper quarter of the stroma.

In two instances, contact inhibition did not take place, and corneal epithelium grew completely around the explant. Both cases were corneas from eyes that contained a uveal malignant melanoma, one from a 68-year-old woman and one from a 78-year-old woman. Both corneas showed evidence of cornea guttata (Fig. 3).

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 for-
Under normal circumstances, epithelium and endothelium do not seem to make contact during wound healing. Abnormal wound healing in man, manifested by uncontrolled migration of corneal epithelium into the anterior chamber (epithelial downgrowth or ingrowth), often results in partial or total loss of vision due to occlusion of the aqueous outflow tract and subsequent increased intraocular tension. Previous studies have shown that after corneal epithelial-endothelial contact is made along the surface of a corneal wound, no further migration of either cell line occurs. The purpose of this study using human corneas 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 agree substantially with in vivo observations in the rabbit and dog. 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, because of 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 is observed relative to this point of contact, and there is 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.

Contact inhibition of epithelial movement has now been demonstrated conclusively for the human cornea as it has for the rabbit and dog. One could speculate therefore 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 the present study, it is shown that diseased endothelium, in the form of cornea guttata, does not inhibit migration of corneal epithelium. It has been suggested previously that manipulation of the corneal flap during delivery of a cataract may traumatize the superior corneal endothelium sufficiently to allow epithelium to grow into the eye and cover the superior two thirds of the posterior cornea, an area which corresponds to the major corneal folding at the time of surgery. 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) might sufficiently modify events at the point of epithelial-endothelial contact as to permit continued epithelial advancement.

Although the present study does not definitely define the problem of epithelial invasion of the eye in human beings, it suggests possible future pathways of investigation. Certainly, the incidence of epithelial downgrowth following intraocular surgery should be compared in eyes with and without cornea guttata.

REFERENCES

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Effect of thimerosal on corneal endothelium

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Widespread use of the mercurial-containing preservative thimerosal as an antibacterial agent in ophthalmic drugs and solutions warranted an investigation into its possible cytotoxic effects on the functional and ultrastructural integrity of the corneal endothelium. No changes in corneal thickness were observed during 5 hours' perfusion of the endothelium of rabbit and human corneas with 0.0001 and 0.0005 percent thimerosal in glutathione bicarbonate Ringer's solution (GBR). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) of the endothelium of the 0.0001 percent group revealed normal ultrastructure. SEM and TEM of the endothelium of corneas perfused with 0.0005 percent thimerosal for 5 hours revealed condensed mitochondria, cytoplasmic vacuoles, and cytoplasmic flaps at the apical end of the cellular junctions. Perfusion of higher concentrations (0.001 and 0.005 percent) of thimerosal in GBR resulted in increases in corneal thickness after 2 hours and irreversible ultrastructural changes.

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