Intercellular Junctions of the Ciliary Epithelium in Anterior Uveitis

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The intercellular junctions of the anterior ciliary and iridial epithelia of the inflamed rabbit eye were examined by use of an ultrastructural tracer, conventional electron microscopy, and the freeze-fracture technique. In normal control eyes, intravascularly injected horseradish peroxidase was prevented from entering the posterior chamber by the zonulae occludentes of the nonpigmented ciliary epithelium. In freeze-fracture studies these junctions appeared as a series of 5-12 branching and anastomosing strands on the P-fracture face, which were complemented by a network of shallow grooves with discontinuous rows of particles at their bases on the E-fracture face. Gap junctions were abundant, particularly between the apical surfaces of the pigmented and nonpigmented layers where they were accompanied by discontinuous tight junctional strands. In eyes inflamed by intravitreal injection of E. coli 055:B55 endotoxin, peroxidase leaked into the posterior chamber primarily from the crests of anterior ciliary and iridial processes. Freeze-fracture electron microscopy of these same areas demonstrated primarily a simplification in junctional complexity and reduction in the number of occluding strands. Severe junctional disorganization and complete junctional fragmentation were rarely seen. A profound reduction in the complement of gap junctions was observed particularly between the apical surfaces of the pigmented and nonpigmented layers. The possible functional significance of the observed changes is discussed. Invest Ophthalmol Vis Sci 28:320-329, 1987

Numerous studies are available in which tracer localization techniques have been successfully combined with freeze-fracture electron microscopy to examine the normal blood-ocular barriers. Combining these techniques has also proven valuable in examining alterations of the blood-retinal barrier stemming from a variety of causes including inherited retinal degeneration and diabetes mellitus.

Three reports are available in which tracer localization studies and freeze-fracture electron microscopy have been used to evaluate the effect of paracentesis on the ciliary epithelium; one examined the monkey and two examined the rabbit. For the latter species, opposite conclusions were reached on whether disruption of the tight junctions between nonpigmented ciliary epithelial cells had occurred.

Although tracer localization techniques alone have been used by several authors to examine the inflamed ciliary epithelium following exposure to prostaglandins or antigen only one recent report is available in which freeze-fracture studies have also been completed.

This article reports the results of combined tracer localization and freeze-fracture studies on the inflamed ciliary epithelium at peak severity of an endotoxin-induced anterior uveitis in rabbits.

Materials and Methods

Animals in this study were used in accordance with the ARVO Resolution on the Use of Animals in Research. New Zealand albino rabbits of either sex, weighing approximately 2 kg, were used. Animals were anesthetized with intravenous pentobarbital (15 mg/kg body wt) supplemented with topical proparacaine-HCl. Specimens were obtained from seven experimental and two control animals. One eye of each control animal received an intravitreal injection of 10 μl of sterile, physiological saline. The opposite eye remained untouched. Experimental animals received intravitreal injections of 1.0 μg of E. coli 055:B55 endotoxin (Sigma Chemical Co.; St. Louis, MO) dissolved in 10 μl of sterile, physiological saline. After 24 hr, each experimental and control animal received an intravenous injection of fluoresceinated horseradish peroxidase (HRP) (250 mg/kg, Miles Yeda Laboratories; Rehovoth, Israel). After 60 min (during which time fluorophotometric studies not reported here were com-
Fig. 1. Unstained electron micrograph of the anterior ciliary epithelium of a control animal. Black HRP reaction product fills the ciliary body stroma (asterisk) and intercellular spaces up to the apico-lateral margin of the "nonpigmented layer". Further diffusion of HRP toward the posterior chamber (PC) is blocked by a tight junction (curved arrow). Note that the intercellular cleft beyond this point is free of HRP (x20,740). INSET: A long curved gap junction is seen to join the apical surfaces of the two epithelial layers (x59,200).

completed) the animals were killed with an overdose of anesthetic. Their eyes were enucleated, opened equatorially, and fixed by immersion in a mixture of phosphate-buffered 2% paraformaldehyde, and 2.5% glutaraldehyde for 3 hr at room temperature. After 10-15 min, the lens was removed from each eye and the anterior uvea was dissected from the corneoscleral tunic in toto. To qualitatively survey uveitis severity, two thin, radial wedges were cut from each specimen and processed for routine paraffin histology. Seven-micron sections were stained with hematoxylin and eosin. For ultrastructural studies the remaining uveal tissues were trimmed into radially oriented wedges from which 150-μ sections were cut with a Smith-Farquhar tissue chopper. Chopper sections were incubated for the demonstration of HRP reaction product as previously described.22 Half of the chopper sections were processed for conventional electron microscopy and half for freeze-fracture.

Conventional Electron Microscopy

Specimens for conventional electron microscopy were postfixed in 1% osmium tetroxide and 1.5% potassium ferrocyanide in distilled water, dehydrated, and embedded in an Epon-Araldite mixture. Light microscopic sections were examined either unstained or stained with toluidine blue. Unstained thin sections and those stained with uranyl acetate and lead citrate were examined with a Philips-300 electron microscope (Eindhoven, The Netherlands).

Freeze-Fracture

For freeze-fracture, chopper sections were individually dissected to obtain specimens from only the crests.
of the iridial and anterior ciliary processes. This was done to minimize the risk that known variations in tight junctional complexity within the epithelium of the ciliary body would be misinterpreted as inflammation-induced changes. Furthermore, previous studies using tracer localization techniques had demonstrated that HRP leakage occurred primarily in these regions of the ciliary epithelium.13

Specimens for freeze-fracture were equilibrated with 20% glycerol in phosphate buffer and mounted on gold specimen carriers. The specimens were rapidly frozen in the liquid phase of partially solidified Freon-22 (monochlorodifluoromethane) and stored in liquid nitrogen. Specimens were fractured and replicated in a Balzer's apparatus (BAF-400; Balzer's High Vacuum Corp.; Santa Ana, CA) operated at approximately 10⁻⁷ torr and a stage temperature of −110°C. The replicated specimens were transferred into methanol overnight and digested in 5% sodium hypochlorite containing 10–15% potassium hydroxide. Platinum/carbon replicas were rinsed with several changes of distilled water and floated onto uncoated copper grids. Replicas were obtained from a minimum of 10-chopper sections per eye and examined with a Philips 300 transmission electron microscope.

**Results**

**Sectioned Material**

In control animals, the intravenously injected peroxidase leaked from the fenestrated vessels of the ciliary body stroma. It permeated the intercellular clefts between adjacent pigmented epithelial cells and between the apical surfaces of the pigmented and nonpigmented layers. HRP was prevented from entering the intercellular clefts between the adjacent nonpigmented cells by zonulae occludentes between the apico-lateral margins of these cells (Fig. 1). Gap junctions were also commonly observed between the cells within each layer and were particularly abundant between the apical surfaces of the pigmented and nonpigmented cells (Fig. 1).

Experimental eyes were moderately inflamed. Their anterior ciliary and iridial processes were edematous primarily near their apices and vascular congestion was evident (Fig. 2). A fibrinous matrix was present in the posterior chambers of these eyes and a moderate, inflammatory cell response was evident (Fig. 2). At the crests of the most edematous processes, the ciliary epithelium often appeared attenuated, but breaks in the continuity of these cell layers were not observed. Intravenously injected peroxidase that filled the stromas of the ciliary and iridial processes was no longer excluded from the posterior chamber by the tight junctions between adjacent nonpigmented cells (Fig. 3).

**Freeze-Fracture**

In uninflamed eyes, nonpigmented epithelial cells of both the anterior ciliary and iridial epithelia were joined by continuous zonulae occludentes. The junctions appeared as branching and anastomosing single and double strands on the P-fracture face, which were complemented on the E-fracture face by a network of shallow grooves with discontinuous rows of particles at their bases (Fig. 4). The junctions varied in complexity; generally 5–12 anastomosing strands occluded the intercellular cleft in each of the two sampled regions.

* Despite the absence of pigmentation in these albino animals, the traditional nomenclature for the two layers of the ciliary epithelium has been retained in order to avoid the use of more confusing terms such as “inner” and “outer.”
In inflamed eyes, alterations in the zonulae occludentes between nonpigmented cells were invariably present. Most often a simplification of junctional complexity and reduction in the number of occluding strands were observed. The apical and basal margins of the junctions were often irregular. Parallel double strands were more evident than in normal eyes, and areas were seen in which the number of occluding strands had been reduced to only one (Fig. 5).

Rarely, profound junctional disorganization was observed. In these areas, short segments of disrupted tight junctional strands were virtually all that remained of the normal zonulae occludentes. Even in these most severely affected areas, a single occluding strand was often tenuously preserved (Fig. 6).

**Gap Junctions**

In replicas of normal ciliary epithelia, gap junctions were commonly observed. These junctions appeared as aggregates of 8–9-nm particles on the P-fracture face, which were complemented by clusters of pits on the E-face. The gap junctions were variable in both size and shape. Junctions composed of only ten particles were often scattered around much larger plaques composed of hundreds of particles (Fig. 7). The largest
complement of gap junctions was found joining the apical surfaces of the pigmented and nonpigmented cells. Here the gap junctions were often accompanied by discontinuous tight junctional strands (Fig. 7).

In replicas of inflamed ciliary epithelium, a striking difference was seen. A marked depletion of gap junctions was observed throughout both epithelial layers. The difference was most pronounced at the apical surfaces of the cells where discontinuous tight junctional strands were seen in the absence of associated gap junctions (Fig. 8).

**Discussion**

Until recently, freeze-fracture studies of abnormal rabbit ciliary epithelium have been limited to examining the effects of paracentesis. Hirsch et al observed no breakdown of the zonulae occludentes in the ciliary epithelium following paracentesis, but no distinctions were made between different areas of the ciliary and iridal epithelia. When such distinctions were made, Yata demonstrated disruption of individual tight junctional strands limited to the crests of the anterior ciliary and iridal processes.

Recently, Noske et al have reported results of combined tracer localization and freeze-fracture studies of the rabbit ciliary body following topical administration of 1 mg of arachidonic acid. Despite this low dose, leakage of HRP across the epithelium of the iridal processes was observed. Freeze-fracture studies demonstrated discontinuities in individual tight junctional strands, particularly on the P fracture face. As was the case in the freeze-fracture studies following paracentesis, however, the generalized and more pronounced reductions in junctional complexity seen in the present study were not observed. Nonetheless, the findings of Yata and those of Noske et al are similar to those in the present study in an important respect. In all three, HRP was seen to have leaked through the intercellular clefts between adjacent nonpigmented ciliary epithelial cells without a disruption of all the tight junctional strands of zonulae occludentes having been present.
Fig. 5. Freeze-fracture replica of inflamed anterior ciliary epithelium. Note the fragmentation of junctional strands and irregularity of the junctional pattern. A reduction in the number of junctional strands is evident. In some locations only a single strand remains (arrow) (×22,256).
These findings suggest that significant increases in blood-aqueous barrier permeability to proteins may occur in the absence of total disruption of tight junctions. Indeed, permeability changes have been reported to occur in several tissues without complete disruption of intercellular tight junctions.23-26

Even among normal tissues, it has been demonstrated that the permeability of the cell layers in which these junctions are found correlates well with the number and complexity of tight junctional strands as shown with freeze-fracture.27,28 It thus becomes reasonable to conclude that total disruption of the tight junctions between ciliary epithelial cells may not be required to elicit an increase in blood-aqueous barrier permeability to proteins. It also raises the possibility that the very small amount of protein normally present in aqueous humor may enter by passing through the less complex regions in the normal tight junctions of the barrier itself.

The finding of large numbers of gap junctions between the cells of the normal ciliary and iridial epithelia is well documented.23 Recently, it has been demonstrated that this type of junction serves to electrotonically couple the ciliary epithelium into a functional syncytium, likely for the purpose of coordinating aqueous secretion.29 The transcellular permeability of gap junctions is sensitive to changes in intracellular pH.
and intracellular levels of free calcium ions. Changes in these parameters, caused by either cell injury or death, lead to a rapid uncoupling of the damaged cells from their neighbors. As an example, a rapid uncoupling of cardiac muscle cells called "healing over" occurs during myocardial ischemia. It is generally held that this response results either from an influx of calcium through the damaged plasma membranes or from a diminished capacity to pump calcium ions out of the cell. The consequence of the attendant loss of coordinated activity in myocardial tissue is self-evident. The functional importance of the observed depletion of gap junctions from the inflamed ciliary epithelium is less clear. Although several other factors are likely involved, it would be consistent to predict that gap junctional uncoupling in the ciliary epithelium would lead to a net reduction in the secretory component of aqueous humor production. It is expected that this response results either from an influx of calcium through the damaged plasma membranes or from a diminished capacity to pump calcium ions out of the cell. The consequence of the attendant loss of coordinated activity in myocardial tissue is self-evident. The functional importance of the observed depletion of gap junctions from the inflamed ciliary epithelium is less clear. Although several other factors are likely involved, it would be consistent to predict that gap junctional uncoupling in the ciliary epithelium would lead to a net reduction in the secretory component of aqueous humor production. It is thus of interest that the depletion of gap junctions found in the inflamed ciliary epithelium occurs at a time in the course of uveitis severity when intraocular pressure is expected to be low.

Key words: intercellular junctions, freeze-fracture, rabbit, anterior uveitis, blood-aqueous barrier, ciliary body

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References