Ultrastructure of the preretinal membrane in retinitis pigmentosa

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Light and electron microscopic examination of the retina and optic nerve in patients with retinitis pigmentosa has revealed a layer of fibrous astrocytes (preretal membrane) apposed to the inner limiting lamina of the optic nerve and retina. This preretal membrane was centered on the disc, was many cell layers thick over the optic nerve and peripapillary retina, and was tapered to a single cell layer near its anterior margin. Processes of fibrous astrocytes from the optic nerve and peripapillary retina extend into the preretal membrane through gaps in the inner limiting lamina of the optic nerve and peripapillary retina. The preretal membrane may be responsible for the abnormally high fundus reflex, the waxy pallor of the disc, and posterior vitreous detachment frequently observed in patients with retinitis pigmentosa.

Key words: retinitis pigmentosa, ultrastructure, preretal membrane, retina, optic nerve

Histopathologic studies of advanced stages of retinitis pigmentosa have shown cellular membranes on the inner surface of the retina and optic nerve. Preretal membranes have also been reported in a number of other disease processes affecting the retina or after traumatic injury to the retina or vitreous. These preretal membranes have been thought to be formed from glial (Müller) cells, pigment epithelium, or astrocytes originating at the site of injury in the retina.

This article describes the ultrastructure and distribution of the preretal membrane in two postmortem donor eyes with retinitis pigmentosa and considers the pathologic findings in the context of clinically visible abnormalities observed in many patients with these diseases.

Materials and methods

Donor eyes were obtained from a 63-year-old female with advanced dominant retinitis pigmentosa and from a 24-year-old male with moderately advanced sex-linked retinitis pigmentosa. The ul-
Fig. 1. Elschnig's layer is seen in a normal retina as a thin cellular membrane over the disc, covering retinal vessels (left arrow) and extending over the peripapillary retina (right arrow). (Calibration bar = 100 μm.)

trastructural findings in photoreceptors and pigment epithelium in these patients have been reported previously. Five eyes from age-matched normal donors were fixed after similar postmortem intervals and were used as controls in this ultrastructural study.

Specimens were prepared for electron microscopy as previously described. In brief, after enucleation the globe was first bisected at the pars plana, and the posterior pole was then immersed in 1% formaldehyde and 2% glutaraldehyde in 0.1M Sorenson's phosphate buffer, pH 7.4, for 2 to 8 hr, washed briefly in buffer, and postfixed in 2% osmium tetroxide in 0.1M phosphate buffer. Light microscopic survey sections were obtained in all quadrants from the optic nerve to the ora serrata and selected areas of the retina were then sectioned for electron microscopy.

Results

A thin cellular membrane, the inner limiting membrane of Elschnig, was seen lying on the inner surface of the optic disc in all normal eyes studied (Fig. 1). It extended over the peripapillary retina about 1 mm or less from the optic disc margin in all quadrants. Elschnig's layer was up to 4 μm thick over the disc and 0.5 μm thick over the peripapillary retina and was composed of multiple layers of flattened and overlapping cell processes of fibrous astrocytes (Fig. 2, A). The processes were filled with numerous filaments 8 to 10 μm in diameter (Fig. 2, B). Vitreous collagen fibrils were closely associated with a thin basal lamina on the inner (vitreal) surface of these fibrous astrocytes (Fig. 2, B).

Elschnig's layer in the donor eyes from patients with retinitis pigmentosa was thicker than that in normal patients (Fig. 3) and was continuous with a preretinal membrane that extended over the retina in all quadrants. The preretinal membrane was centered on the disc and had a diameter of 9 mm in the donor with sex-linked retinitis pigmentosa and of at least 18 mm in the donor with advanced dominant retinitis pigmentosa. The preretinal membrane varied in thickness in both patients; it was 15 to 17 μm over the disc and had a diameter of 9 mm in the donor with sex-linked retinitis pigmentosa and of at least 18 mm in the donor with advanced dominant retinitis pigmentosa. The preretinal membrane varied in thickness in both patients; it was 15 to 17 μm over the optic nerve (Fig. 3), 6 to 10 μm thick over the peripapillary retina (Fig. 4, A) and macula (Fig. 4, B), and 1 to 4 μm over the fovea (Fig. 5, A) and at its most anterior border (Fig. 5, B).

The preretinal membrane was composed of overlapping layers of cells with long flattened cell processes (Figs. 3 to 7). The cell bodies generally contained many mitochondria, Golgi apparatus, and much of the endoplasmic reticulum in each cell (Fig. 6, B), and the processes were filled with numerous 8 to 10 μm filaments (Fig. 6, B). Junctions of the macula adherens type were generally found on the inner surface at the tip of long cell processes.

The preretinal membrane rested on the basal lamina of Müller cells (inner limiting
Fig. 2. A, Fibrous astroglial cells in Elschnig's layer (E) form a thin cellular membrane between the vitreous (V) and the adventitia (A) of retinal vessels on the surface of the optic nerve (ON). B, Cell body and processes of fibrous astroglial cells in Elschnig's layer are densely filled with fine 8 to 10 µm filaments. A thin basal lamina (arrows) is found at the vitreal surface (V) and also adjacent to the adventitia (A) of a retinal vessel. Small diameter vitreal collagen and large diameter adventitial collagen appear to be closely associated with the basal lamina. (Calibration bar = 2 µm, A; 1 µm.)
Fig. 3. A, Elschnig’s layer (E) is considerably thicker than normal (compare with Fig. 1) at the edge of the optic nerve (ON) in the donor eye with sex-linked retinitis pigmentosa. B, Elschnig’s layer (E) lies adjacent to the inner limiting lamina (arrows) of the optic nerve in the donor eye with dominant retinitis pigmentosa. The nuclei of fibrous astroglial cells (F) are seen in the retina and in Elschnig’s layer. (Calibration bar = 20 μm, A; B, 5 μm.)
Fig. 4. A, Preretinal membrane (P) is seen over the peripapillary retina in sex-linked retinitis pigmentosa. Remaining cones (C) in this region have lost outer segments, and the inner segments are apposed to the pigment epithelium (PE). B, Multilayered preretinal membrane (P) is apposed to the inner limiting lamina (arrow) of the retina in the macular region in dominant retinitis pigmentosa. (Calibration bar = 20 μm, A and B.)
Fig. 5. A, Section through the fovea of a patient with sex-linked retinitis pigmentosa shows the preretinal membrane (P) artifactually detached from the surface of the retina. B, Section from the temporal midperipheral retina of a patient with advanced dominant retinitis pigmentosa. Receptors are absent and there is considerable disorganization of the retina with accumulation of bone spicule pigment (arrows). The anterior limit of the preretinal membrane (P) has tapered to a thin single cell layer in this region and lies over the irregular surface of the retina (Calibration bar = 20 μm, A and B.)

Discussion

The ultrastructural findings in this study of the preretinal membrane in retinitis pigmentosa have shown that the preretinal membrane is continuous with Elschnig's membrane, originates from fibrous astroglial cells in the optic nerve, is symmetric around the optic nerve, and forms an unbroken cellular layer over the optic nerve and retina. The
Fig. 6. A, Inner limiting lamina (I) is seen between the preretinal membrane (P) and the foot processes of Müller cells (M) in a patient with sex-linked retinitis pigmentosa. Some collagen (arrows) is seen between fibrous astroglial cells in the preretinal membrane. B, Processes of fibrous astroglial cells in the preretinal membrane are filled with fine 8 to 10 μm filaments. Vitreal collagen (V) is closely apposed to the thin basal lamina of the fibrous astroglial cell. The preretinal membrane is apposed to the inner limiting lamina (I). (Calibration bar = 1 μm, A and B.)
Fig. 7. Processes of fibrous astrocytes (F) are seen crossing a gap in the inner limiting lamina of the retina (defined by the arrows) to become part of the preretinal membrane (P) in the peripapillary retina of a patient with advanced dominant retinitis pigmentosa. G, Ganglion cell axons; N, nuclei of fibrous astrocytes. (Calibration bar = 2 μm.)

degenerative changes in photoreceptors in these patients with retinitis pigmentosa were symmetric around the fovea, corresponding closely to the patients’ constricted fields. Therefore it seems unlikely that the preretinal membrane contributed in a primary way to their photoreceptor degeneration.

In normal eyes, Roth and Foos demonstrated glial connections with Elschnig’s layer via breaks in the inner limiting lamina of the optic nerve and peripapillary retina. Preretinal membranes have also been shown to originate from glial proliferation through breaks in the inner limiting lamina at the site of localized injury to the retina. In contrast, breaks in the inner limiting lamina were seen only over the optic nerve and adjacent peripapillary retina in these cases of retinitis pigmentosa. Large areas of retina in the patients with dominant disease and sex-linked disease were found where photoreceptors had degenerated and considerable gliosis was present, although these areas were not covered by a preretinal membrane. The absence of breaks in the inner limiting lamina over the retina and the absence of a preretinal membrane over areas of advanced retinal gliosis in retinitis pigmentosa would suggest that the astroglial cells forming the preretinal membrane did not proliferate from localized sites of degeneration and gliosis but arose from the optic nerve and peripapillary retina through breaks in the basal lamina over the optic nerve and peripapillary retina. The symmetric distribution of the preretinal membrane around the optic nerve also supports the idea that it originates from the optic nerve.
Continuity between Elschnig's layer and preretinal membranes has not been previously demonstrated in idiopathic preretinal macular fibrosis or after injury to the retina. In contrast, in retinitis pigmentosa the fibrous astrocytes of Elschnig's layer were in direct continuity with and indistinguishable from the fibrous astrocytes forming the preretinal membrane.

In normal young patients the vitreous body is attached firmly to the inner limiting lamina in a narrow zone in the peripapillary retina. However, anterior vitreous detachment per se in normal subjects does not appear to result in the subsequent appearance of a preretinal membrane. Vitreous degeneration and posterior vitreous detachment have been described in retinitis pigmentosa as early as the first decade of life. The possibility can be raised that the outgrowth of fibrous astrocytes from the optic nerve and their insinuation between the inner limiting lamina and vitreous body from its peripapillary attachment. However, it remains to be determined whether a preretinal membrane forms prior to the appearance of posterior vitreous detachment in early stages of retinitis pigmentosa.

Many patients with retinitis pigmentosa, when examined with an ophthalmoscope, have an abnormally bright reflex that appears to emanate from near the vitreal surface of the retina. It would appear that a preretinal membrane composed of multiple layers of randomly oriented overlapping cell processes filled with densely packed fibers as seen in these studies could create an optically reflective layer on the surface of the retina, giving rise to this high retinal reflex. An apparent waxy pallor of the disc is also seen in many patients with retinitis pigmentosa. In studies of experimental optic atrophy, pallor of the optic disc was shown to be related to axonal loss and reorganization of astrocytes. It would appear that reorganization of fibrous astrocytes in the optic nerve and migration of fibrous astrocytes in the abnormally thick Elschnig's layer over the optic nerve could sufficiently alter the transmission and reflection of light from the disc to contribute to the waxy pallor of the disc seen in many patients with these diseases.

REFERENCES

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