Clinical-Ultrastructural Study of a Retinal Dystrophy

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An ultrastructural and cytochemical study was performed on the retina and retinal pigment epithelium of an eye surgically enucleated for choroidal melanoma from an otherwise healthy 31-year-old man. The patient and his identical twin show a retinal dystrophy that, based on clinical appearance, visual fields, and electrophysiology, is most likely autosomal recessive retinitis pigmentosa. Rod and cone photoreceptors were reduced in numbers and outer segments were virtually absent in the region corresponding to the patient’s poorest vision. In the region from approximately 20° to 60° (best field of vision), the outer segments of rods and cones were shortened and disorganized. The retinal pigment epithelium showed reactive changes in areas of most severe photoreceptor pathology, including reduplication, loss of melanin, increased melanolysosomes, and migration of individual cells into the retina. The acid phosphatase reactivity of both the retinal pigment epithelium and photoreceptor cells appeared normal, as were the photoreceptor cilia and inner layers of the retina. This study thus provides improved ultrastructural documentation of a relatively early case of retinitis pigmentosa that may provide a foundation for further functional studies aimed at elucidation of this enigmatic retinal dystrophy. Invest Ophthalmol Vis Sci 24:458–469, 1983

Recent ultrastructural studies of retinitis pigmentosa (RP) have been performed upon postmortem specimens of either moderately advanced sex-linked RP1 or advanced, presumed autosomal dominant RP.2,3 Although the previous studies have provided new ultrastructural information on poorly understood retinal dystrophies, each was compromised by postmortem autolytic changes2 or previous prolonged exposure of the patient donor to cytotoxic drugs for treatment of malignant disease.1,3 This report presents the ultrastructure and cytochemistry of the retina and retinal pigment epithelium of an eye surgically enucleated for choroidal melanoma from an otherwise healthy 31-year-old man who, together with his identical twin brother, shows a retinal dystrophy. Based on the clinical appearance, visual fields and electrophysiology, the most likely diagnosis in our patient and his identical twin is autosomal recessive RP.

Materials and Methods

The propositus, a 31-year-old white man of French and English origin had noted delayed dark adaptation for at least 9 years. At age 29 a choroidal tumor was found in the superior temporal quadrant of the left eye during a periodic eye examination. Over the next two years the mass increased markedly in size. Our first examination of July 25, 1979 showed visual acuity of the right eye 20/20 and the left eye 20/100, with correction of moderate myopic astigmatism. The refraction was: right eye, —3.50 + 1.25 X 142; left eye, —4.50 + 2.75 X 30. The eyes appeared normal except for the fundi, which both were lightly pigmented and showed midperipheral bone corpuscular retinal pigment alterations (Fig. 1). The peripheral macula in the area of the temporal vascular arcades in each eye showed many faint, cream-colored deep retinal dots that were approximately 50–100 μm in diameter. The optic discs appeared normal, and the retinal vessels appeared normal or very slightly narrowed.

The left eye showed a moderately pigmented choroidal tumor in the temporal periphery. The mass was highly elevated and was more than 10 mm in diameter. The inferior fundus was occupied by a retinal detachment with subretinal fluid that shifted superiorly to reach the fovea and the inferior margin of the mass when the patient assumed the recumbent position.

Goldmann perimetry (Fig. 2) showed a dense ring scotoma in the right eye. There was advanced visual...
field loss in the left eye due to the retinal detachment but some inferior field was preserved. Electroretinography⁴ of the right eye (Fig. 3) showed prolonged latency and decreased amplitude of the b-waves to single light flashes in both the dark-adapted and light-adapted state. In the dark-adapted state, there was no detectable response to a single blue flash; a single white flash had a b-wave latency of 48.4 msec (predicted 47.7 msec) and a b-wave amplitude of 75.0 μV (predicted 586.4 μV). A flickering white light at 30 Hz gave a b-wave latency of 34.8 msec (predicted 29.2 msec) and a b-wave amplitude of 36.6 μV (predicted 84.6 μV). A single white flash in the light-adapted state gave a b-wave latency of 30.8 msec (predicted 28.8 msec) and a b-wave amplitude of 29.4 μV (predicted 122.4 μV).

The patient and his twin brother were identical for both HLA A and B antigens, 16 blood group antigens, and 19 red blood cell antigens. The patient's twin brother had noted delayed dark adaptation for 9 years, and a diagnosis of retinal dystrophy had been made at another institution. Our examination showed visual acuity of 20/20 in each eye with correction of myopic astigmatism. The refraction was: right eye, -4.25 + 1.75 × 155; left eye, -6.25 + 3.50 × 30. The ocular fundi were similar to that of the propositus with the exception of the tumor and retinal detachment. Visual fields and electroretinographic findings were indistinguishable from those of the right eye of the propositus.

The patient's father was 70 years old and his mother, 65 years old. Neither had evidence of retinal dystrophy by history, fundus examination, or visual field testing. His parents were unavailable for further evaluation. The patient's two brothers and three sisters had no visual problems, nor did the seven sons of his sisters. Two maternal uncles had no history of visual difficulty. The patient's only child was an 18-month-old boy who was not examined. The patient's twin had no children.

Because of the increasing size of the tumor in a visually compromised eye, enucleation of the left eye of the propositus was performed on August 16, 1979. The cornea was slit, the vitreous chamber was injected through the pars plana with 2% paraformaldehyde-2% glutaraldehyde in 0.18 M phosphate buffer, and the globe was placed immediately into the same fixative. Both attached and detached regions of the retina were processed by routine procedures for electron microscopy. Semi-thin (1 μm) sections were stained with Richardson's mixture; thin sections (1000 Å) were stained with uranyl acetate and lead citrate and examined with an AEI 801 or JEOL 100S electron microscope. Following overnight fixation, some samples were washed overnight in 10% sucrose in phosphate buffer, tissue chopped at 40 μm and reacted for the cytochemical demonstration of acid phosphatase.⁵ Control specimens were processed identically with omission of the B-glycerophosphate substrate. Following osmication, the specimens were processed for electron microscopy as above.

Results

Gross Examination

The globe appeared normal with the exception of a moderately pigmented mass (15 mm in diameter × 11 mm high) arising from the choroid in the superior temporal quadrant and a retina with fine, bone corpuscular pigment that was detached inferiorly and temporally to the level of the fovea and inferior margin of the mass.

Microscopic Examination

Histologically the choroidal tumor was comprised of spindle B cells with prominent nucleoli; there was no scleral extension. The fovea was detached and showed the typical ultrastructure of retinal detachment,⁶ including loss of photoreceptor outer segments and migration of pigment and lipid-laden macrophages into the fluid-filled subretinal space. The photoreceptor layer was reduced to a single layer of cone inner segments, most of which appeared normal, although a few had swollen mitochondria. The cone pedicles were atrophic with few synaptic vesicles and ribbons. The inner layers of the detached retina appeared normal but contained occasional pigment-laden macrophages in the inner nuclear layer. The
optic nerve head and choroid appeared normal, and no preretinal membrane was present.

In the attached region of the retina extending out to approximately 20°, which corresponded to a region of severe loss of visual field, rod and cone inner segments were present in reduced numbers, but outer segments were, for the most part, absent. Occasional cone cells had very disorganized outer segments that were much shorter (2.0 μm long) (Fig. 4) than cone outer segments (approximately 12 μm) found in this region of normal retinas (Bunt-Milam, unpublished). In scattered foci the retinal pigment epithelium (RPE) was reduplicated, usually with depigmentation of the scleral layer, and a pigmented RPE cell and/or macrophage appeared to be invading the retina.

In the region of the retina from approximately 20°
Fig. 3. Electroretinography showed prolonged implicit times and decreased amplitudes for the right eye. All values are abnormal at a confidence level of at least 98% for our laboratory based upon means and values predicted from age-matched normal subjects. A, Blue flash, dark-adapted (normal, 189.9 μV). B, White flash, dark-adapted (normal, 586.4 μV). C, 30 Hz flicker in darkness (normal, 84.6 μV). D, White flash, light-adapted (normal, 122.4 μV).

to 60°, corresponding to the patient’s field of best vision, rods and cones had shortened outer segments with disorganized lamellae (Fig. 5). By light microscopy, the longest rod and cone outer segments found were 5 μm and 2.5 μm, respectively. Occasional phagocytosed outer segment tips were found in the RPE (Fig. 5, inset). Apical pyramid-shaped protruberances of the RPE were filled with melanin granules and enclosed individual short cone but not rod outer segments (Fig. 6). This appeared to represent an accentuation of the microvillus sheaths normally found around individual cone outer segments of normal length.

The inner segments of the rods had swollen mitochondria and electron dense inclusions free in the cytoplasm with granular, heterogeneous contents (Figs. 7, 8), which were also prominent in the Müller cell perikarya. An occasional rod inner segment (Fig. 9) appeared degenerate as evidenced by cytoplasmic densification, as well as glycogen inclusions. The cone inner segments contained the normal complement of organelles (Fig. 10), and the connecting cilia of the rods and cones (Figs. 10, inset, 11), and the RPE (Fig. 10) appeared normal morphologically.

The far periphery of the retina contained very few photoreceptor cells and showed hypertrophy of the Müller cells (Fig. 12). The rods and cones lacked outer segments, although some inner segments appeared normal. Macrophages that had paler cytoplasm than the RPE cells and contained ingested melanin granules were found in the inner layers of the retina as well as among the RPE cells (Fig. 13). Often a macrophage had a nuclear inclusion of medium electron density that was generally irregular in shape (Fig. 12). The external limiting membrane was prominent and contiguous to the apical surface of the RPE (Fig. 14).
Fig. 5. Electron micrograph from retina at approximately 50° eccentricity. Note short, disorganized cone outer segment but normal appearing inner segment (IS). The adjacent rod inner segment (R) contains swollen mitochondria but the retinal pigment epithelium (RPE) appears normal (×12,560). Inset: phagocytosed outer segment tips in the RPE (×26,400).

An occasional cell in the RPE was necrotic (Fig. 14).

The RPE cells from all regions showed normal acid phosphatase activity and distribution, most prominently in the Golgi apparatus, in lysosomes, and at the periphery of melanin granules in which the linear matrix was recognizable (Fig. 15). The photoreceptors also showed the normal distribution of acid phosphatase in the Golgi region and occasional autophagosomes. The granular, electron dense inclusions in the rod inner segments were negative for acid phosphatase (Fig. 16). No enzyme activity was present in
Fig. 6. Retina as in Figure 5. Electron micrograph illustrating apical pyramidal-shaped protruberances of the retinal pigment epithelium which are filled with melanin and enclose individual cone but not rod outer segments (×20,000).

Discussion

The diagnosis of RP in our patient and his monozygotic twin is based on the clinical finding of progressively abnormal dark adaptation, ring scotomata on visual field testing, abnormal rod and cone responses on ERG, and typical fundus appearance. The mode of inheritance may be autosomal recessive. However, the advanced age of the patient's father at the time of his birth and the relatively mild clinical involvement are compatible with a new mutation for an autosomal dominant gene. Similarly, transmission of an X-linked recessive gene from the phenotypically normal mother cannot be excluded.  

The ultrastructural findings correlate well with the patient's visual fields, in that in the region of loss of
Fig. 7. The inner segments of the rods have swollen mitochondria and electron dense, granular inclusions (arrows). C, connecting cilium (×8,610).

Fig. 8. Higher magnification of granular inclusions in a rod inner segment (×26,630).

Fig. 9. A, Dense rod inner segment which contains glycogen (G) and dense, granular inclusions (*) (×10,030). B, Higher magnification of glycogen (G) (×26,630).
Fig. 10. A cone inner segment appears normal morphologically but lacks an outer segment and abuts the retinal pigment epithelium. Note hypertrophy of Müller cell processes (×6490). Inset: Cross section of normal appearing connecting cilium of cone at higher magnification (×54,360).

Fig. 11. Longitudinal section of normal appearing rod connecting cilium and basal body. The rod outer segment membranes (OS) are disorganized (×44,950).
Fig. 12. The far periphery of the retina contains very few photoreceptor cells (R, rod inner segment) and shows hypertrophy of the Müller cells (M). A macrophage with pale cytoplasm, pigment granules and characteristic nuclear inclusion (*) is found in the photoreceptor layer (×7,840).

vision due to RP (from fovea to 20°), the photoreceptors were decreased in number and had virtually no outer segments. The few remaining outer segments were small and disorganized, although all connecting cilia examined were morphologically normal. This stands in contrast to the suggestion\(^1\) that RP might represent one expression of a generalized cilium defect affecting other organs as well as the retina. In the region of the retina from approximately 20° to 60°, corresponding to the patient’s field of best vision, both rods and cones had outer segments, although they were shortened and disorganized. The rod inner segments contained abnormal, electron dense inclusions that are similar but not identical to lipofuscin.\(^1\) These deposits also resemble those interpreted as autophagosomes in RP retinae,\(^1\) but the deposits here were negative for acid phosphatase, suggesting that they are not components of the lipofuscin or lysosome-autophagosome system. The acid phosphatase activity of the RPE cells appeared normal, although the number of melanolysosomes seemed higher than normally found in RPE cells at this age.\(^1\) In contrast to some previous studies,\(^13\)\(^14\) there was no evidence of extracellular acid phosphatase around the degenerating photoreceptors; this is in agreement with the cytochemical findings of Essner and Gorin\(^1\) on the dystrophic retina of the RCS rat.

Pigmented cells of two types were found invading

Fig. 13. A pale macrophage lies in the vitread layer of reduplicated retinal pigment epithelium. N, pigment epithelium nucleus; M, macrophage nucleus (×7,430).

Fig. 14. Very rarely a necrotic cell with melanin granules free in the cytoplasm was found in the retinal pigment epithelium. E, junctions of external limiting membrane (×6,770).
Fig. 15. A–B, Retinal pigment epithelium which has been reacted for the demonstration of acid phosphatase activity. Note reaction product in lysosomes (L) and in interior of melanin granule (M) in which linear matrix is recognizable. Some melanin granules (double arrows) are rimmed by reaction product (A, ×15,660; B, ×16,660).

Fig. 16. Tissue processed as in Figure 14. Note positive acid phosphatase reaction in Golgi apparatus (G) of retinal pigment epithelium and absence of reaction in dense, granular inclusion (arrow) of rod inner segment. M, Müller Cell microvilli; IS, swollen mitochondria in rod inner segment (×15,800).

the retina. Some appeared to be typical RPE cells that were in the process of migrating away from the RPE layer. Others resembled macrophages with a paler cytoplasm and clusters of melanin granules within phagosomes. The macrophages may be local or hematogenous in origin or represent RPE cells that have undergone metaplasia. The RPE showed other reactive changes, including islands of reduplication and loss of melanin, particularly in the scleral layers. These islands of reduplication appeared to corre-
spond in size (~50–100 μm) and location to the clinically observed, cream-colored deep retinal dots. No other abnormal structures of the appropriate size and distribution could be found that might correspond to these deep dots.

These observations of a well-preserved specimen of RP from an otherwise healthy 31-year-old man corroborate previous suggestions16 that the disease results from a primary defect in the rod and cone photoreceptors. The outer segments in this case are severely affected, although relatively normal inner segments appear to persist, with some actual loss of photoreceptor somata. Morphologically, the RPE does not appear to be affected initially, appearing for the most part normal in the region from 20° to 60° where the photoreceptor outer segments show pathologic changes. Of course, a primary metabolic defect in the RPE might not be manifest there initially but rather be expressed early on as abnormal photoreceptor morphology and even photoreceptor death. The changes observed in the RPE in the more central and far peripheral retina (reduplication and migration into the retina) may possibly be reactive in nature, perhaps in response to significant photoreceptor death. This study thus provides improved ultrastructural documentation of a relatively early case of RP that may provide a foundation for further functional studies aimed at elucidation of this enigmatic retinal dystrophy.

Key words: retinal, dystrophy, electroretinogram of retinitis pigmentosa, inherited human retinal dystrophy, retinitis pigmentosa ultrastructure

Acknowledgments

The authors are grateful to David M. Smith, MD, for providing this specimen, D. F. Milam, MD, and M. J. Reeh, MD, for histopathologic consultation, R. Patterson, I. Klock, and D. Ichikawa for technical assistance, J. Foltz and B. Clifton for photographic help, and J. Seng for secretarial assistance.

Addendum

Following acceptance of this manuscript for publication, the patient’s mother consented to be examined. Her fundi showed no carrier signs of retinitis pigmentosa. A corneal, full-field ERG, using the same technique employed to evaluate the proband, showed no abnormalities in the dark-adapted state to single blue, red, or white flashes. In the light-adapted state there was mild increase in B-wave latency to single flashes of white light (34.48 msec [31.05 msec expected]), yellow-red light (31.39 msec [27.88 msec expected]), and blue green light (31.76 msec [23.32 msec expected]). These ERG changes are not typical of the majority of women who are heterozygous for the X-linked retinitis pigmentosa gene as reported by Berson et al. (Am J Ophthalmol 87: 460, 1979) who found abnormalities of B-wave amplitude to white light (dark-adapted) in 19 of 23 obligate heterozygotes. We continue to believe that our patient most likely has the autosomal recessive form of retinitis pigmentosa.

References