Progressive Retinal Atrophy in the Abyssinian Cat

Electron Microscopy

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Seven adult Abyssinian cats at different stages of a recessively inherited retinal degenerative disease (progressive retinal atrophy) were studied ultrastructurally. At the stage of early disease, in 2-yr-old cats, disorganized and vesiculated discs were found in less than half of the rod outer segments in the periphery, while similar changes were seen in the central retina only infrequently or in patches. Cones appeared normal in all areas of the retina at the early stage. With progression of disease, the lesions were more advanced in all areas of the retina, and involved both rods and cones, with the most severe alterations found in the midperiphery. At the advanced stage, in a 6-yr-old cat, both rods and cones were lost, the inner nuclear layer thus being separated from the pigment epithelium by Müller cell processes and a few remaining outer plexiform processes only. Remnants of photoreceptor outer and inner segments, macrophages, and what appeared to be displaced photoreceptor cell nuclei could be found occasionally in the subretinal space, however. Clumps of pigment granules were often observed in the photoreceptor layer in the non-tapetal fundus. The pigment epithelium remained morphologically intact as a single layer of uninterrupted cells throughout the disease process, as did tapetal cells and choriocapillaris. There was no difference in the severity of disease between the peripheral tapetal and non-tapetal fundus. In the inner retina, only minor alterations were observed. These changes appeared at a later time than photoreceptor degeneration, and were considered secondary to the latter. Invest Ophthalmol Vis Sci 27:1569-1576, 1986

A recessively inherited retinal disease has been described in a strain of Abyssinian cats.1-3 The retina is usually ophthalmoscopically normal until the age of 1.5-2 yr, although changes occasionally appear earlier. Alterations are seen as grayish discolorations, most evident in the midperipheral and peripheral tapetal fundus. With progression of the disease, the discoloration becomes generalized, and in 2-4 yr degenerative changes are severe; these include tapetal hyperreflectivity and vascular attenuation, as well as decoloration and, sometimes, hyperpigmented spots and streaks in the non-tapetal fundus.

In a previous paper, the clinical characteristics of the sequential stages, as seen ophthalmoscopically, of the retinal degeneration found in these cats were described.4 Light microscopic and electrotetroretinographic findings were correlated to the disease stage. Results from this clinical study, as well as from more extensive electrophysiologic investigations, reported separately,5 indicated a primary photoreceptor disorder which could serve as a useful model for human retinitis pigmentosa (RP).

The present study concerns a description of the disease process at the ultrastructural level. Furthermore, some of the abnormalities found previously in ophthalmoscopy and light microscopy will be described and discussed in more detail based on the electron microscopic results.

Materials and Methods

The cats included in this study were all adult Abyssinians (Table 1), euthanized upon request by the owners, because of problems unrelated to the retinal disease. Within 2 min after death, the right eye from seven affected and two ophthalmoscopically normal Abyssinian controls were enucleated. The eye was hemisected at the ora serrata, and the posterior eye-cup was fixed by immersion in a cold solution of 2% glutaraldehyde (pH 7.2) in 0.1 M sodium cacodylate buffer. Fixation was continued at 4°C for 1-3 weeks. The eye-cup was then cut in smaller pieces under a dissecting microscope either before or after post-fixation in 1% osmium tetroxide in Veronal acetate buffer. Pieces, about 3 x 5 mm, from three locations within the tapetal
area (the posterior pole—temporally to the disc, the midperiphery, and the periphery) as well as from the peripheral non-tapetal area, were dehydrated and embedded in Vestopal W. For light microscopy, 1 μm sections were cut and stained with toluidine blue. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 300 electron microscope. These investigations conformed to the ARVO Resolution on the Use of Animals in Research.

Results

Early Stage of Disease

In comparison to the normal controls, abnormalities were found in less than half of the rod outer segments in the peripheral retina at the stage of early disease; no alterations were found in cones (Figs. 1, 2). Affected outer segments showed severe disorganization of lamellae and vesiculation. Stacks of lamellae were disoriented, giving the outer segment an irregular or sometimes even a collapsed appearance. These alterations usually were observed along the entire length of the affected outer segment. In several otherwise normal appearing rod outer segments, numerous vesicular-like structures were observed at the base, encompassing the space of up to 20 lamellae (Fig. 3). Small vesicles at this location may be seen also in rods from normal cats, although they are not as numerous as in affected cats. Inner segments were usually normal, except for some distended vacuoles, possibly swollen mitochondria or autophagic vacuoles; these changes were often found in conjunction with a degenerating outer segment. Phagocytic cells containing debris and melanin granules were occasionally observed in the subretinal space (Fig. 4). The outer nuclear layer was reduced in width from 7–9 rows to 3–5 rows, and sometimes degenerating nuclei were observed among normal-appearing ones. The pigment epithelium and choriocapillaris were normal. So-called dense bodies or lysosomal bodies, as well as phagosomes, were observed in the pigment epithelium. No alterations were seen in the inner retina, including the photoreceptor synapses (Fig. 5).

In the midperiphery, alterations were as described above, or somewhat less pronounced at this early stage.
Fig. 2. The peripheral non-tapetal retina of cat A1 at the stage of early disease. A severely disorganized rod outer segment (*) is seen with disrupted lamellae and vesiculation. A vacuole (arrow), possibly a dilated mitochondrion or an autophagic vacuole, is shown in the corresponding inner segment. A cone (C) and other rods (R) appear normal. (×12,900)

Centrally, there were areas where most photoreceptors appeared unaltered. Infrequently, however, degenerating rod outer segments could be observed as solitary lesions or in patches. The photoreceptor nuclei were normal-appearing, although the outer nuclear layer was somewhat reduced, from 9–13 rows to 8–9 rows.

Moderately Advanced Stage

At this stage, retinal alterations were more advanced in all areas of the retina, being most severe in the midperiphery and affecting both rods and cones (Fig. 6). In this area, no normal-appearing photoreceptors could be observed. Affected rod and cone outer segments were
severely disorganized. Some were disrupted, leaving more or less free remnants of photoreceptor lamellae in the subretinal space. Inner segments were short and stubby. Degenerative changes in the latter ones were seen as an increase in electron density, swollen or disrupted mitochondria (Fig. 6), or complete lysis of entire inner segments. Occasionally, displaced stacks of outer segment lamellae were observed in inner segments or even in the outer nuclear layer. Clumps of melanin granules, as well as phagocytic cells with or without pigment granules, were sometimes observed in the subretinal space or in the outer nuclear layer (Fig. 7). Pyknosis was seen among normal-appearing photoreceptor nuclei (Fig. 6), more frequently at this stage than at the early stage. The pigment epithelium still appeared normal. Apical microvilli were abundant, enclosing degenerating outer segments.

In the periphery, alterations were somewhat less severe. Only occasionally were shortened but otherwise normal-appearing photoreceptors still present among degenerating ones. In the central retina, photoreceptors were more preserved, although often somewhat shortened in this area also.

**Advanced Stage**

Alterations were generalized with severe lesions in all areas of the retina at the advanced stage. Most of the photoreceptors were lost, and the external limiting membrane was in direct apposition to the pigment epithelial processes (Fig. 8). Occasionally in the central...
retina, but also in the periphery, a few receptor nuclei were still present, although they had an irregular configuration or were pyknotic. In these areas, the subretinal space was occupied by sparse remnants of outer and inner segments, macrophages, and, sometimes, displaced photoreceptor cell nuclei.

Proliferation of Müller cells was extensive, showing a widespread network of cell processes extending from the inner retina to the external limiting membrane. Such extensions were also seen to intermingle with apical microvilli. The pigment epithelial cells remained morphologically normal, forming a single layer of uninterrupted cells. As in the normal cats, they were narrow only where capillaries indented the cell base. Sometimes, pigment epithelial cells appeared slightly more electron-dense than at earlier stages, and an increased amount of rough-surfaced endoplasmic reticulum could be seen. Tapetal cells retained their normal configuration and regularity (Fig. 8). Synapses were lacking in the outer plexiform layer. In the inner nuclear layer, some bipolar cell nuclei appeared slightly more electron dense than usual, and irregular in shape. No other alterations were observed in the inner retina.

There were no differences with respect to the severity of degenerative changes between the peripheral tapetal and non-tapetal fundus at any stage of the disease process as seen by electron microscopy. In the cases where pigment migration was observed, it was recognized only in the non-tapetal area or at the border between the tapetal and non-tapetal fundus.
Fig. 7. Melanin granules, possibly in a phagocyte or a Müller cell, are seen in the outer nuclear layer of the non-tapetal retina at a stage of moderately advanced disease (cat A6). (x9,300)

Discussion

The hereditary retinal disease described in this strain of Abyssinian cats is a slowly progressive degenerative process of the rod and cone system. In many ways, it is similar to human RP. Just as in the latter, the rods in the midperipheral and peripheral retina are most severely affected at first; then, both rods and cones degenerate in a successive manner from the equatorial zone towards the central retina.6-11

Early changes in the disease were lamellar disorientation and vesiculation, most often along the entire length of certain rod outer segments. Minor alterations were also found in corresponding rod inner segments. These findings, together with the loss of visual cell nuclei already at an early stage of disease, indicate that entire rods are lost early in the disease.

Cones seemed to have an increased tolerance as compared to rods in the disease process. At the early stage, degenerating rod outer segments were observed, while cone outer and inner segments appeared normal. In electroretinographic studies reported on previously,5 normal cone flicker responses were obtained at the stage of early disease, demonstrating a normal cone function, while rod function (scotopic b-wave) was already impaired. Although there seemed to be a greater proportional persistence of cones, both types of receptors were, however, affected morphologically at later stages.

Aberrant cells were observed in the subretinal space, namely macrophages and photoreceptor nuclei. The macrophages, containing debris and sometimes pigment granules, could be dedifferentiated pigment epithelial cells, or possibly of hematogenous origin.12-13

Despite the marked degeneration of the visual cells, the pigment epithelium retained its normal morphology, even at the advanced stage of disease. The cells contained normally distributed cellular organelles, suggesting a preservation of its normal functions. The dense appearance of the cytoplasm of some pigment epithelial cells, as well as the increased amount of rough-surfaced endoplasmic reticulum, could indicate considerable metabolic cell activity. However, it cannot be excluded that a slight cell shrinkage may also be involved in causing a dense appearance.

The gray discoloration seen ophthalmoscopically in certain areas of the tapetal fundus, always observed in conjunction with early and moderately advanced disease, seemed to correspond mainly to degenerative lesions in the photoreceptor cell layer; disorganization and disruption of photoreceptor outer and inner segments and the occurrence of aberrant cells. The retinal atrophy seen ophthalmoscopically as hyperreflectivity in the tapetal area at the end stage of the disease corresponded to the loss of visual cells, with the external limiting membrane often in direct apposition to the pigment epithelium. The hyperreflectivity seems to be explained by the retinal thinning, with tapetal cells getting nearer to the incident light. The generalized decoloration of the non-tapetal fundus, observed clinically in some affected cats at a late stage, appeared to be explained by the loss of photoreceptor cells and, possibly, by some loss of melanin granules in the pigment epithelium (counting of such granules was not performed, however). Through breaks in the cell membrane of the epithelial cell processes, melanin granules could be liberated and scattered in the visual cell layer. The hyperpigmented spots observed in the decolored non-tapetal zone could be clumps of free melanin granules, or granules within the cytoplasm of macrophages.

Several strains of animals with hereditary retinal degenerations are used in research on visual cell disease.14,15 These include the RCS rat, the rd, rds, and pcd mice, the Rhode Island red chicken, and the Miniature Poodle, Norwegian Elkhound, Collie, and Irish setter dog breeds. It has been shown that the mode of inheritance, the pathogenesis, and the etiology often vary in different animal models. The retinal degenerations in these animals have been divided into two basic groups, according to the stage of retinal differentiative process at which lesions occur.16,17 Either the photoreceptors degenerate during their differentiative phase or very soon thereafter, or degeneration occurs after their differentiation. Examples of the first type of degeneration are the defects found in the rd mouse18 and in the Irish setter19 and Collie20 dog breeds. The late onset type of degeneration found in the pcd
Fig. 8. Advanced stage. Central tapetal retina (cat A7). The external limiting membrane (arrows) is in apposition to the pigment epithelium (PE) from which long apical microvilli are extending. Degenerative debris is seen between the outer plexiform layer and the pigment epithelium (double arrow). The inner nuclear layer appears normal. (×3,900)

mouse and in the Miniature Poodle exemplifies the second type of diseases.

Recently, an early-onset type of retinal disease with an autosomal dominant mode of inheritance has been described in a group of Abyssinian cats in England, different from the strain presented here. Clinically, nystagmus and dilated pupils were observed in kittens, and an advanced retinal degeneration was already seen ophthalmoscopically at the age of 12 weeks.

The retinal disease in this strain of Abyssinian cats is a slowly progressive degeneration of the rod and cone systems, with a late onset of clinical symptoms. Thus, the retinas are usually ophthalmoscopically normal until the age of 1.5 yr, with normal pupillary responses until late in the disease process. Areas of normal-appearing photoreceptors are seen ultrastructurally, especially in the central retina, at the early stage of disease. Preliminary developmental studies in two young Abyssinians, homozygous for the defect, have, however, already shown some ultrastructural alterations at the age of 7 and 12 weeks, respectively (unpublished observation). Although the retinas of these
Kittens were ophthalmoscopically normal, electron microscopy demonstrated lesions that were comparable to those described for what is termed “early stage of disease” in the present paper, where staging was based upon ophthalmoscopy.

The retinal structures of the cat are essentially morphologically complete by the age of 4 weeks.25 Thereafter, the process of maturation in the cat retina is rather slow. The retina continues to develop structurally and functionally until at least 12 weeks postnatally, but does not fully attain adult characteristics until after the age of 4 months.25,26 Future studies will be directed towards establishing when in the process of retinal maturation degenerative lesions first appear, analyzing the model with respect to possible biochemical defects, and also explaining why some photoreceptors degenerate rather early, while others that seem to have differentiated and matured in a normal way eventually degenerate.

Key words: progressive retinal atrophy, retinal degeneration, Abyssinian cat, morphology, ultrastructure, electron microscopy

Acknowledgments

The authors are indebted to Ms. Marianne Nordell and Ms. Christel Daliri for excellent technical assistance.

References