Age-Related Changes of the Retinal Pigment Epithelium of Cats With Chediak–Higashi Syndrome

Linda L. Collier,* Edward J. King,† and David J. Prieur‡

The eyes of 7, 9, and 11-year-old Chediak–Higashi syndrome (CHS)-affected cats were examined by light, fluorescence, and electron microscopy. Numerous round to oval bodies of various sizes were associated with the retinal pigment epithelium (RPE). These bodies stained positively with periodic acid–Schiff. They also displayed a bright yellow autofluorescence and stained positively with a prolonged Ziehl–Neelsen acid-fast method for demonstration of lipofuscin, suggesting that they contained lipofuscin or a lipofuscin-like material. Ultrastructural examination disclosed the bodies to be secondary lysosomes and large to giant-sized residual bodies. Many of the residual bodies were extracellular and formed drusen-like mounds, covered by deposits of basal lamina, beneath the RPE. Also evident were scattered degenerated RPE cells and other RPE cells that had detached and migrated into the interphotoreceptor space. The presence of drusenoid bodies, and the loss of cells from the RPE monolayer in CHS eyes have not been reported previously. Many of the changes in the CHS cat eyes resemble those in non-CHS aging eyes of man and other species. Invest Ophthalmol Vis Sci 27:702–707, 1986

The Chediak-Higashi syndrome (CHS) is an autosomal recessive genetic disease of man, cats, and four other species.1 Enlarged cytoplasmic granules, such as secondary lysosomes and melanosomes, are characteristic of the syndrome.2 Hypopigmentation and giant melanin granules have been observed in CHS human eyes.3–6 Ultrastructural abnormalities in the retinal pigment epithelium (RPE) of two CHS infants and one child have been described also.7,8

Hypopigmentation and enlarged melanosomes are evident at the light microscopic level in the eyes of young adult CHS cats.9 The eyes of CHS affected kittens are hypopigmented, and giant melanosomes and complex granules consisting of melanosomes within lysosomal matrix are present in the RPE.10 The tapetum lucidum cellulosum degenerates in CHS kittens.11

The effects of longterm abnormality of the lysosomal and melanosomal systems on the RPE of CHS individuals have not been reported previously. In this study we examined the eyes of aged CHS cats to determine the morphologic consequences of the CHS defect having been present over a period of years. Previously unreported structures included extracellular, autofluorescent, drusen-like giant residual bodies at the bases of RPE cells; these bodies were covered by basal lamina. Loss of RPE cells through degeneration of scattered individual cells and detachment of others was also evident.

Materials and Methods

The animals were raised and maintained in standard animal care facilities with cyclic illumination and were treated in full compliance with the ARVO Resolution on the Use of Animals in Research. The eyes of CHS cats aged 7, 9, and 11 years, and control cats aged 8 and 12 years were obtained for microscopic examination. The eyes of the 7- and 11-year-old CHS cats were enucleated and fixed within 1 hr after deaths (several hours after light onset) of the animals that resulted from pneumonia or renal infection. These eyes were fixed in 10% neutral buffered formalin. The other cats (9-year-old CHS, and the 8- and 12-year-old controls) were anesthetized deeply with sodium pentobarbital and the eyes enucleated 3 hr after onset of morning light. The right eyes were processed for paraffin embedment for light microscopy and the left eyes were processed for embedment in Epon–Araldite (epoxy) resin for light and electron microscopy.

Light Microscopy

Whole globes were fixed by immersion in Zenker’s fixative or formalin. The lateral calottes were removed
from each eye and the remaining central portion was embedded in paraffin and sectioned at 5 μm. Each section was stained with one of the following stains: hematoxylin and eosin (HE), periodic acid-Schiff (PAS) with and without diastase digestion, or prolonged Ziehl–Neelsen acid-fast stain for demonstration of the oxidized lipids present in lipofuscin. Additional unstained sections from each eye were deparaffinized, then examined by darkfield fluorescence microscopy using a mercury lamp with a UG 1 excitation filter having maximum transmission at 365 nm and a 450 nm cutoff filter. One-micron thick, toluidine blue stained sections of epoxy embedded eyes were also examined by light microscopy. Measurements were made by use of a calibrated ocular micrometer.

Electron Microscopy

The eyes were bisected, the vitreous bodies removed, and the eye cups immersed for at least 4 hr in paraformaldehyde–glutaraldehyde fixative at room temperature. Vertical strips were cut from the superior edge to the inferior edge of each eye cup through its center, then subdivided into specimen blocks that were embedded in Epon–Araldite. The blocks were numbered to permit comparison of specimens from corresponding areas of each eye. Ultrathin sections were examined and photographed in a JEM-100B electron microscope.

Results

Light Microscopy

The appearance of the control cat eyes was typical of adult cats. The neuroepithelium (with the exception of that portion of the RPE overlying the tapetum lucidum) and the choroid were uniformly heavily pigmented with small, dark brown melanin granules. When the unstained control eye sections were illuminated by ultraviolet light, a faint, diffuse, yellow autofluorescence was visible in the RPE.

The CHS cat eyes were hypopigmented in both the neuroepithelium and the uvea. The melanin present in the RPE was in the form of giant granules, most of which were located in the peripheral RPE. No melanin was present in many of the RPE cells in the inferior portion of the fundus, whereas comparable RPE cells located in the nontapetal fundus in normal cats were heavily pigmented. In the toluidine blue-stained sections of epoxy-embedded tissues, the RPE and choroidal melanin granules were various shades of blue, green, gold and brown, rather than the uniform brown observed in the control eyes.

Numerous pale brown bodies, 5–20 μm in diameter, at the level of the RPE were evident in the HE-stained paraffin sections of the CHS eyes. These bodies were round, oval, or irregular and were more numerous and tended to be larger in the posterior poles of the eyes than in the peripheral RPE. The bodies stained positively with PAS and prolonged Ziehl–Neelsen histochemical stains. With these staining methods, many smaller positively stained inclusions, ranging in size from 1.25–5 μm, were also evident in the RPE. In unstained sections of CHS eyes illuminated by ultraviolet light, the RPE bodies displayed a yellow autofluorescence similar to that characteristic of RPE lipofuscin (Fig. 1A).

In the epoxy sections, numerous round to oval bodies, corresponding in size to those observed in the paraffin sections, were present in the RPE. The staining characteristics of the bodies varied greatly, with the colors ranging from pale blue, almost unstained, to dark blue. The appearance of the contents of the bodies varied from homogeneous to heterogeneous granular, and melanin granules were incorporated into some of the bodies in the peripheral RPE (Figs. 1B, C). The bodies varied greatly in size, with some extending from the bases to the apices of, or even distorting the RPE cells (Figs. 1B, C). Most of the small to medium-sized bodies were intracellular inclusions, but some of the larger bodies elevated the RPE in a manner resembling drusen (Fig. 1C). The drusenoid bodies appeared to be extracellular and located on Bruch's membrane, with basal lamina evident between the inclusions and the RPE cells.

Loss of cells from the RPE monolayer had also occurred in the CHS cat eyes. Individual degenerated RPE cells were scattered among cells that were not degenerated. Many of the degenerated cells were swollen, pale-staining, and most contained enlarged, round, pale nuclei (Fig. 1B). In addition, widely scattered individual cells (averaging two cells per paraffin embedded section) were present in the interphotoreceptor space. Some of these cells may have been macrophages that were present to remove debris released from disintegrated, degenerated RPE cells, but many appeared to be RPE cells that had detached from the monolayer (Fig. 1D).

Since the majority of the CHS RPE cells did not contain melanin, it was not possible to positively identify some cells in the inner retina as migrated RPE cells. In most of the paraffin sections, two to three heavily pigmented cells that may have been migrated RPE cells or macrophages were present at the levels of the inner plexiform layer and inner nuclear layer in the peripheral retina adjacent to the ora ciliaris retinae. Greater spacing was present between CHS nuclei than control nuclei in the RPE cell layer. In the posterior poles of the CHS eyes, 52 nuclei per 0.5 mm were present, in contrast to 87 nuclei per 0.5 mm in the control eyes.
Fig. 1. The retinal pigment epithelium (RPE) of a 9-year-old CHS cat. A, Autofluorescent giant drusenoid (arrow) and RPE inclusion bodies (arrows). The fluorescent bodies were bright yellow. The autofluorescence of the photoreceptors and choroidal erythrocytes was pale yellow-green, distinctly different from that of the RPE bodies. Paraffin-embedded tissue, unstained, 5-μm thick section (×450). B-D, Epoxy-embedded tissue, toluidine blue stained, 1-μm thick sections. B, A degenerated cell is evident as well as inclusions (arrows) of various sizes and staining intensities (×1640). C, Drusen-like, giant residual bodies (arrows) beneath the RPE. Material was present in each of the inclusions, but could not be demonstrated photographically in some inclusions. An RPE cell with its nucleus was displaced into the interphotoreceptor space (×740). D, Detached RPE cell in the interphotoreceptor space. Inclusions and melanosomes are evident within the cell. The RPE monolayer was distorted by a large inclusion (arrow), and smaller inclusions (arrows) are also visible. Bruch’s membrane (arrowhead) appears thickened (×1640).

Electron Microscopy

Structures corresponding to the PAS positive, autofluorescent bodies of the CHS RPE were large secondary lysosomes and large to giant-sized residual bodies. Some of the largest bodies were actually extracellular and surrounded by deposits of basal lamina material (Fig. 2A). The heterogeneous contents of the giant residual bodies included membrane whorls, vesicular structures resembling remnants of cellular organelles, and discrete particles of material of varying sizes, shapes, and electron densities. In the peripheral RPE, various forms of melanosomes, many of which appeared to be degenerated, were also found in the giant residual bodies.

Each melanin granule that was not incorporated into a giant residual body was surrounded by a thin layer of secondary lysosomal matrix. A dark, dense layer,
often with an irregular surface was present around the peripheries of most melanin granules, just inside the lysosomal layer. The centers of some of the melanin granules were less dense than normal and some had a fibrillar appearance and appeared to be degenerated (Fig. 2B).

Alterations in other structures were also observed. Basal infoldings were absent or rare in the CHS RPE cells (Figs. 2A, B). The RPE basal lamina appeared thickened and discrete deposits of material, with the appearance and staining characteristics of new basal lamina, were present in many of the RPE cells (Fig. 2B). In addition, phagosomes were commonly seen in the basal portions of many of the RPE cells of the CHS cat at 3 hr post light onset (Fig. 2B), in contrast to control cat RPE, in which phagosomes were rarely seen at the same phase of the light cycle.

**Discussion**

The most obvious abnormal structures of the CHS cat RPE were the giant residual bodies. Enormous residual bodies have not been reported previously in CHS eyes, probably because of the relatively young ages of the humans and animals studied. The contents of these structures in the cats were heterogeneous and appeared to consist of cellular debris, membrane whorls and fragments, and, in the peripheral RPE, melanosomes. Of particular interest were the similarities between many of the CHS giant residual bodies and some of the drusenoid bodies observed in non-CHS human eyes.\(^7\)\(^,\)\(^4\)\(^,\)\(^3\) There were similarities in position of the bodies, as well as in the apparent reaction of the RPE cells in covering the bodies with a thick layer of basal lamina. Numerous abnormally large inclusions that stained positively with PAS and had the ultrastructural appearance of secondary lysosomes were also evident in the CHS cat RPE. Large secondary lysosomes have also been reported in the RPE of two CHS children\(^7\) and of middle-aged CHS mice (100–200 days old).\(^16\) Although discrete granules of the size, shape, and electron density typical of human RPE lipofuscin granules\(^17\) were not evident in the CHS cat RPE, the giant residual bodies and large secondary lysosomes appeared to contain lipofuscin or lipofuscin-like material. These structures autofluoresced similarly to lipofuscin and stained positively by a prolonged Ziehl–Neelsen method for the demonstration of lipofuscin. Increased accumulations of lipofuscin have also been reported in the RPE of CHS mice.\(^18\) The giant residual bodies and large secondary lysosomes contained membrane whorls and vesicular membranes, many of which probably were derived from phagosomes, whereas others may have originated as RPE intracellular membranes. Degradation of the membranes appeared to have been impaired. A membrane defect has been reported in CHS, involving an increase in unsaturated fatty acid content.\(^19\)\(^,\)\(^20\) This could lead to increased peroxidation of both phagosome-derived and intracellular CHS RPE membranes, making them resistant to lysosomal degradation and contributing to accelerated accumulation of autofluorescent debris in secondary lysosomes and residual bodies in the CHS RPE.

Loss of melanin from the RPE also occurred with age in the CHS cat eyes. Many of the melanin granules appeared to be degenerating in the secondary lysosomes and giant residual bodies. Material that appeared to be fragments of melanin granules also was present in the giant residual bodies. Degenerating melanosomes within RPE lysosomes have been reported in two CHS children as well.\(^7\)

Also noteworthy, and not reported previously in CHS eyes, was the obvious loss of RPE cells from the RPE monolayer through both death of some cells and detachment and migration of other RPE cells. Degeneration and loss of RPE cells also occurs with aging\(^21\) and with age-related maculopathies\(^22\) in human eyes.

In addition to similarities to drusen formation and lipofuscin accumulation, as well as loss of melanin and RPE cells, there were other changes in the CHS cat RPE resembling those in aging non-CHS eyes of man and other species.\(^21\)\(^–\)\(^24\) These included decreased numbers and altered structure of basal infoldings, as well as deposits of discrete mounds of new basal lamina material. Thickening of the basal lamina and Bruch’s membrane may also occur in CHS. The CHS cats used in this study were middle-aged for domestic cats; thus, accelerated aging appears to be a consequence of the CHS basic defect.

Further investigations, including cytochemical and quantitative ultrastructural studies of the eyes of younger adult CHS cats, are currently underway to determine the sources of material in the giant residual bodies as well as the morphologic sequences involved in development of the drusenoid bodies and other changes that occur with aging in CHS eyes.

**Key words:** Chediak–Higashi syndrome, cats, RPE, aging, drusen, lipofuscin

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**References**