The Ocular Pigmentary Disturbance of Human Chédiak-Higashi Syndrome

A Comparative Light- and Electron-microscopic Study and Review of the Literature

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The ultrastructure of the ocular pigment abnormality of human Chédiak-Higashi syndrome is described. The presence of giant abnormal melanosomes, probably the end result of the fusion of smaller abnormal organelles, was the most striking pathologic finding. This defect involved both optic cup and neural crest-derived melanocytes; the former were affected more severely. Giant lysosome-like organelles were also observed. (Key words: Albinism; Photophobia; Pigmentation; Melanosomes.)

CHÉDIAK-HIGASHI SYNDROME (CHS) is a rare autosomal recessive disorder characterized by oculectaneous pigmentary dilution, photophobia, nystagmus, great susceptibility to infections, and the presence of large cytoplasmic inclusions, considered abnormal lysosomes, in the bone marrow and peripheral leukocytes as well as in various tissue cells. A similar disorder occurs in Aleutian mink, beige mice, Hereford cows, killer whales, and cats. Lipid-like material has been reported to accumulate in the lysosomes of CHS mice. During the "accelerated phase" of the disease, generalized lymphohistiocytic infiltrates develop with hepatosplenomegaly, lymphadenopathy, and pancytopenia. Although the ocular histologic changes of the human disease have been studied previously by light microscopy, to our knowledge, the ultrastructural features have never been reported. It is the purpose of this paper to describe them in comparison with those of normal eye. In addition, the pertinent literature will be reviewed.

Report of a Case

The general clinicopathologic features of this case have been reported in detail. Only a brief summary will be included.

Clinical Findings. A 2-month-old black male infant was admitted to the hospital because of irritability and fever. Physical examination revealed albinoid skin, "blonde" hair, and hepatosplenomegaly. Ophthalmologic examination showed minimal development of the eyebrows, pigmented eyelashes, photophobia, inconstant slow pendular horizontal nystagmus, slate-grey irises, and pigmented fundi. Abnormal laboratory findings included a marked Coombs' positive hemolytic anemia, neutropenia with lymphocytosis, thrombocytopenia, severe ferropenia with a saturation of 6%, and typical large cytoplasmic inclusions, morphologically and cytochemically diagnostic of CHS, in the peripheral blood and bone marrow leukocytes.

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Therapy with corticosteroids, vincristine, blood transfusions, iron, and antibiotics was followed by a remarkable, though temporary, clinical improvement. During the next months, the patient had several severe febrile episodes of unknown cause. His condition progressively deteriorated (accelerated phase), and his death, seven months after his initial admission, was preceded by hemorrhage and the reappearance of thrombocytopenia and Coombs'-positive hemolytic anemia.

Pathologic Findings. The skin was microscopically devoid of melanin. Nonlymphomatous lymphohistiocytic infiltrates were present in lymph nodes, bone marrow, spleen, liver, kidneys, adrenal glands, stomach, myocardium, and lungs. Giant periodic acid-Schiff (PAS)-positive cytoplasmic inclusions were identified in neurons, glial cells, choroid plexus epithelium, Kupffer's cells, infiltrating histiocytes, thymocytes, and renal tubular epithelium. Electron-microscopic study showed large abnormal lysosome-like organelles in neutrophils, lymphocytes, histiocytes, thymocytes, hepatocytes, Kupffer's cells, and renal tubular epithelium.

Materials and Methods

CHS Patient

The eyes with attached distal optic nerve segments were removed at autopsy one hour after death. The right eye was fixed in 10% neutral formalin. After fixation, appropriate horizontal samples were dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin according to the usual histologic procedures for light-microscopic examination. Special stains of paraffin-embedded blocks included PAS (with and without diastase digestion), alcian blue, Fontana-Masson, Prussian blue, ferric ferricyanide, Crimbe, and treatment with K MnO₄ for melanin bleaching. Tissue frozen sections were also stained with Sudan black B.

For electron-microscopic examination, samples from retina, choroid, ciliary body, and iris were trimmed into 1-mm cubes and fixed in 4% paraformaldehyde solution buffered with 0.1 M sodium cacodylate (pH, 7.4) for two hours, washed in 0.1 M sodium cacodylate containing 0.1 sucore, postfixed in 2% osmium tetroxide in the same buffer, dehydrated in ascending series of ethanol solutions, and embedded in Spurr® plastic. The resulting blocks were cut with a glass knife on a Porter-Blum® MT-2 ultramicrotome. One-micrometer sections were stained with 1% toluidine blue and examined by light microscopy to pick out representative areas. Ultrathin sections of selected blocks were obtained with a DuPont® diamond knife on the same microtome, mounted on 220-mesh copper grids, stained with 1% uranyl acetate and lead citrate; the sections were studied with an RCA-EMU® 3H electron microscope.

Control Patient

As a control for histologic comparison, normal eyes of a 2-week-old black male infant were fixed and processed (including special stains) for light and electron microscopy in the same manner as that described. This patient was born in the forty-second week of a normal gestation; the postnatal period was complicated by asphyxia, generalized bleeding, and renal failure.

Results

For the description of electron-microscopic findings, we will follow the melanosome terminology used by Mund and associates.

Light Microscopy

No melanin was seen in the basal and suprabasal layers of the conjunctival epithelium. The iris exhibited a moderate decrease in stromal pigmentation. The pigmented cells contained numerous small melanin granules morphologically similar to those of a normal eye. Most of the cells of the epithelial layer were nonpigmented. The occasional pigmented cells contained single giant spherical-to-oval dense melanin granules that filled most of the cytoplasm (Fig. 1). The melanin content of the ciliary epithelium was also reduced in both pars plana and pars plicata. Where present, however, the melanin granules were mostly of the giant type described. The epithelial cells of the retina were vacuolated and displayed an almost complete lack of pigmentation. Only a few pigmented cells with enlarged granules were seen. Papilledema and overt lymphohistiocytic infiltrates frequently described in previous reports were not present.

Histochemistry

Both large and small melanin granules stained black with Fontana-Masson and bleached after treatment with K MnO₄. In addition, some apigmented epithelial and stromal cells contained large spherical diastase-resistant PAS-positive cytoplasmic inclusions (Fig. 2). These also stained positively with Sudan black B, alcian blue (Fig. 3), and ferric ferricyanide. Occasionally, on specially stained blocks, melanin-like granules of various sizes deposited in the center of these inclusions were seen (Figs. 2 and 3).

The control eye showed normal melanin pigmentation in both epithelial and stromal melanocytes (Fig. 4) without any evidence of the large cytoplasmic inclusions described above.

Electron Microscopy

Electron-microscopic examination of retina, ciliary body, iris, and choroid revealed single giant spherical-to-oval mature melanosomes (melanin granules) (Fig. 5). The largest ones were comparable in size to that of
FIG. 1 (upper, left). Chédiak-Higashi syndrome. Hypopigmented iris with occasional giant melanin granules in epithelial cells (E). The stroma is also hypopigmented. Melanin pigmentation is better preserved at the anterior border layer (B). Hematoxylin and eosin. x 140.

FIG. 2 (upper, right). Chédiak-Higashi syndrome. Large PAS-positive granules in cells of the ciliary body stroma (arrowheads). Precipitation of melanin pigment is seen in some of them (P). Note the contrast between amelanotic melanocytes and the presence of giant spherical-to-oval melanin granules in others. Periodic acid-Schiff. x 600.

FIG. 3 (lower, left). Chédiak-Higashi syndrome. Giant round alcian-blue-positive spherical granule in the hypopigmented epithelium of pars plana of the ciliary body (arrowheads). Note deposition of melanin pigment (P) in the center of the granule. Alcian blue. x 600.

FIG. 4 (lower, right). Iris of control eye showing normal pigmentation in both epithelium (E) and stroma. Hematoxylin and eosin. x 140.

the melanocyte nucleus and were found mainly in the epithelial melanocytes. Occasionally, the peripheral portions of these giant organelles were unmelanized (late immature melanosomes) (Fig. 6) and exhibited a finely granular slightly electron-dense matrix, instead of the fibrillar one usually found in the normal immature melanosomes of the normal eye (Fig. 7).

The stromal melanocytes were also affected to a lesser degree. They contained abnormal large melanosomes that varied in size and shape.

In addition, some epithelial and stromal melanocytes revealed giant spherical-to-oval lysosome-like organelles. These organelles were made up of finely granular, slightly electron-dense matrices and were bound by a unit-membrane. Occasionally they contained central cores of electron-dense homogeneous melanin-like pigment (Fig. 8). They seemed to correspond to the PAS-positive inclusions seen by light microscopy. Occasionally, fusion of smaller organelles was observed. No lymphohistiocytic cells were identified in the areas examined.

The control eye showed normal, late immature, and mature melanosomes in both stromal and epithelial melanocytes (Fig. 9), without evidence of the giant membrane-bound organelles described above.

Comments and Review of the Literature

Although CHS was first described in 1943, the ocular histologic findings were not reported until 1957, when choroidal hypopigmentation as well as infiltration of the choroid and optic nerve by leukocytes was mentioned in a brief communication. Three years later, Spencer and Hogan reported the results of the first comprehensive study. They observed a remarkable decrease in pigmentation in the anterior border layer and pigment epithelium of the iris, choroid, ciliary epithelium, and pigment retinal epithelium, although some melanin-containing cells were present in these areas. Papilledema and infiltration of the choroidea and optic nerve by immature lymphocyte-resembling cells were also identified. Later, three additional reports sub-
FIG. 5 (upper). Chédiak-Higashi syndrome. Pigment iridal epithelium with single giant spherical-to-oval mature melanosomes (P). Compare with Figure 9. Their size is similar to that of the cell nucleus (N). Cell basement membrane and characteristic villous cytoplasmic projections are seen at the right lower corner. x7,400.

FIG. 6 (lower, left). Chédiak-Higashi syndrome. Huge immature melanosome in the ciliary body epithelium. Melaninization occurs upon a finely granular matrix (G) that seems to correspond to the PAS- and alcian blue-positive areas. White arrows outline a denser core within the melaninized portion. R, rough endoplasmic reticulum; T, tight junction. x16,200.

FIG. 7 (lower, right). Control eye. Normal immature (IM) and mature melanosomes in the ciliary body epithelium. Arrowheads indicate a normal cross-sectioned partially melaninized fibrillary matrix. Compare with the abnormal granular one of Figure 7. x16,200.

stantiated the ocular melanin anomalies described and added some new features. An evident contrast between the lack of melanin in the ocular pigment epithelium and the somewhat preserved uveal melanin content was observed in one case, and occasional melanin granules found in the retinal epithelium were described as very large in another. The inflammatory infiltrates were described as lymphocytic, histio-
Fig. 8 (upper). Chédiak-Higashi syndrome. Membrane-bound giant lysosome-like organelles (arrowheads). Deposition of electron-dense pigment is seen in the left lower one (P). Electron-dense spheres are present along the limiting membrane of the larger one (E). Fusion of two organelles seems to be occurring at F. R, two dilated endoplasmic reticulum; T, tight junction; M, swollen mitochondrion. x 14,000.

Fig. 9 (lower). Control eye. Numerous mature melanosomes, normal in size and shape, in the pigment epithelium of the iris. N, cell nucleus. x 7,400.

cytic, or lymphohistiocytic. Papilledema was present in two cases.

Of special interest is the 1969 paper by Bedoya and co-workers; they described abundant large melanin granules in the iris, ciliary body, and choroid. The retina showed cellular vacuolization. The optic nerve was normal, and apparently no inflammatory cells were visualized.

In this case, the pigmentary ocular abnormality involved both the epithelial and stromal melanocytes, but the latter were affected to a lesser degree. Ultrastructurally, the most striking feature of the ocular
melanocytes was the aggregation of melanin into abnormal giant mature melanosomes. Some immature giant melanosomes revealed a peripheral un melanized, finely granular abnormal matrix, and some amelanotic cells showed giant spherical lysosome-like organelles without evidence of the usual melanosomal matrix, containing central cores of electron-dense melanin-like pigment. These giant organelles revealed an abnormal lipoprotein content, as suggested by our histochemical findings. Similar lipid material was also present in the abnormal lysosome-like organelles of other organs. Although histoenzymatic studies were not performed in this case, because of the unavailability of fresh ocular tissue, the ocular hypopigmentation in patients who have CHS is probably related to an ultrastructural melanosomal defect, rather than to an inefficient tyrosine-tyrosinase system as it occurs in classic albinism. The accumulation of lipid material in these giant organelles may be a reflection of the metabolic abnormality that underlies CHS. Perhaps a common biochemical defect could lead to the abnormal formation of both melanosomes and lysosomes. The photophobia and nystagmus are probably expressions of this ocular pigmentary dilution.

Giant melanosomes have also been described to occur in the skin of patients who have CHS. However, in this case, we could not document a similar cutaneous abnormality, since no melanin was detected in several abdominal skin sections (including special stains), and no cutaneous melanosomes were identified by electron microscopy.

The hypopigmented eyes of CHS mink and mice have also been described; they contain very large melanin granules. A reduced amount of ocular pigment has been also documented histologically in CHS cattle. It has been suggested, on the basis of ultrastructural histochemical studies, that the giant granules in the retinal pigment epithelium of the beige mouse arise from fusion of primary lysosomes with melanin granules that are already enlarged from previous fusion among melanosomes. The African white-tailed rat also has an abnormal ocular pigmentation characterized by the presence of prominent complex melanin-containing granules. However, this animal does not exhibit the characteristic cytologic hallmark of CHS.

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References

5. Essner E, Oliver C: Lysosome formation in hepatocytes of mice with Chediak-Higashi syndrome. Lab Invest 30:596–607, 1974