Mucolipidosis IV: Ocular, systemic, and ultrastructural findings

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The ocular and systemic findings in four children with mucolipidosis IV (ML IV), a new variant of mucolipidosis, are described. Corneal clouding from birth or early infancy is a prominent feature in all of the patients and in two of them, this was the presenting symptom. Psychomotor retardation usually does not become apparent until the end of the first year of life. Conjunctival biopsies revealed two types of abnormal inclusion bodies: (1) single-membrane-limited cytoplasmic vacuoles containing both fibrillogranular material and membranous lamellae, and (2) lamellar and concentric bodies similar to those found in Tay-Sachs disease. The abnormal cytoplasmic organelles were present in both the stromal fibroblasts and the epithelial cells. The electroretinogram performed in one patient was subnormal.

Key words: mucolipidoses, conjunctival biopsy, corneal clouding, psychomotor retardation, mucopolysaccharidoses, ultrastructure, cytoplasmic vacuoles, lamellar storage bodies.

The mucolipidoses have been classified as a distinct, albeit heterogeneous, group of storage diseases having many biochemical, ultrastructural, and clinical features overlapping both the mucopolysaccharidoses and sphingolipidoses. In some of the mucolipidoses, ocular manifestations are common and may include corneal clouding, macular grayness, or a cherry-red spot. There are presently 10 confirmed disorders classified as mucolipidoses, and recently a new variant has been described in which congenital corneal clouding is a prominent finding. Since this first description, three more patients with similar clinical signs have been diagnosed by us; we have suggested the name of mucolipidosis IV (ML IV) for this new inherited systemic disorder. For the ophthalmologist, this variant is especially important because moderate to severe corneal clouding often precedes the relatively mild systemic manifestations. The purpose of this communication is to describe the ocular findings in ML IV, and to stress the importance of ultrastructural abnormalities of the conjunctiva in the diagnosis of this new syndrome.
Fig. 1. Photograph of patient No. 4 (MS) at the age of 11 months.

Case reports

Patient No. 1. This male child (AR), was the first reported case of ML IV. He was the third child in a family of Jewish-European (Ashkenazi) origin. The parents are unrelated and the two older brothers are normal. At the age of six weeks the mother noticed bilateral corneal clouding and the child was referred for further examinations. General physical and neurologic functions were normal at that time. However, mild motor retardation was first noticed at about the age of one year.

Slit lamp examination revealed severe corneal cloudiness in the form of multiple opaque dots and short lines throughout all layers of the stroma. The haziness was diffuse and homogeneous from center to periphery. The epithelium did not seem to be involved and there was no edema. The bulbar and palpebral conjunctiva appeared normal. The fundus, which could be seen only with the strong light of the indirect ophthalmoscope, was normal. Intraocular pressure was normal. Re-examination at the age of two years showed that the corneal opacity had become more pronounced. The fundus was only barely visible by this time.

Patient No. 2. A female (KV) was the first child of healthy unrelated Jewish-European (Ashkenazi) parents. At the age of eight months the mother noticed a squint and some corneal cloudiness in addition to mild psychomotor retardation. Although the child began to sit at 11 months, and to stand up at 13 months, she was unable to walk alone by the age of two years. Her vocabulary consisted of about four words.

An eye examination performed at the age of eight months showed that the corneal cloudiness had become severe and that the fundus could be seen only by strong light. The fundus was normal. The child had a high myopia of approximately -12.0 diopters in both eyes, and a squint which, after treatment by patching, turned into an alternating esotropia.

Patient No. 3. A female (AZ) was the first child of healthy unrelated Jewish-European (Ashkenazi) parents. At six months of age, the parents noticed slowness in motor development and by the age of one year, definite signs of mental and motor retardation were present. The child was unable to sit up by the age of 30 months, the time of the last examination. There was hypotonia of all four extremities with increased tendon reflexes and ankle clonus. At the age of 18 months, the eyes were examined for the first time and bilateral corneal cloudiness was found. Slit lamp biomicroscopy showed that the opacities were located in the stroma and were relatively mild. The child had an alternating esotropia. A detailed clinical report of this patient will appear elsewhere.

Patient No. 4. A female (MS) was the third child of healthy unrelated parents of Jewish-European (Ashkenazi) origin (Fig. 1). Signs of mental and motor retardation were apparent from a rather early age. She was unable to raise her head until the age of nine months, and only by 14 months could she sit in a bucket seat. The corneal cloudiness was found at the age of eight months. Slit lamp examination revealed that the opacity was due to an epithelial bedewing. The stroma seen through the edematous epithelium seemed only slightly affected. The child had an alternating convergent squint and bilateral epicanthal folds. The fundus seemed normal.

Materials and methods

Enzyme assays. These were performed on cultured fibroblasts using the same method as described previously.

Light and electron microscopy. After local anesthesia by Novesine, small biopsy specimens of bulbar conjunctiva were obtained from an area about 4 mm. from the limbus at the six o’clock position. The specimens were immediately cut and fixed according to one of the following methods: (1) primary fixation in 5 per cent glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for two hours, followed (after washing) by post-fixation in 2 per cent osmium tetroxide in phosphate buffer for 40 minutes at 4° C, and (2) fixation in 2 per cent osmium tetroxide, buffered at pH 7.4 with 0.1 M phosphate buffer, for 1.5 hours at 4° C.
All specimens were dehydrated in graded alcohols and propylene oxide and embedded in Epon 812. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined in a Philips 300 electron microscope.

Histological examination of 1μ sections by light microscopy was performed after staining with 1 per cent toluidine blue in borate buffer.

Electroretinography (ERG). This was performed on patient No. 2 (KV) after sedation, local anesthesia, and dilation of the pupils. The electrodes used were a Henkes contact lens over each eye, a reference electrode at each mid supraorbital rim, and a ground electrode on one ear lobe. The electroretinograms (ERG's) were evoked by single light stimuli using intensities I0 to I4, at a distance of about 20 cm. The preamplifiers had filters open from 0.1 to 2,000 cycles per second.

Visual evoked potentials (VEP). The visual evoked potential (VEP) was recorded on a cathode-ray oscilloscope as the averaged response of 70 light stimuli applied at a frequency of about one per second. The responses were derived bipolarly between a silver disc electrode at the inion and another electrode 5 cm. above.

Results

Laboratory findings. Serum urea, glucose, calcium, phosphorus, alkaline phosphatase, transaminase, electrolytes, and protein were normal in all four patients. Serologic tests were all negative. Routine urinalyses, as well as amino acid chromatography and spot tests for excess mucopolysaccharides, were negative. Quantitative analysis of urinary mucopolysaccharides revealed normal values. Radiologic surveys of the skeleton, electroencephalography, and chromosomal analyses were all normal. Bone marrow aspirates showed varying degrees of abnormalities in all four patients. Numerous enlarged histiocytic-like cells were present, and the cell cytoplasm contained many small vacuoles. The activities of 10 acid hydroases in cultured skin fibroblasts were within normal limits as compared to control subjects. The enzymes examined were N-acetyl-β-glucosaminidase, β-galactosidase, N-acetyl-β-D-galactosaminidase, α-L-fucosidase, α-galactosidase, β-glucuronidase, α-mannosidase, β-glucosidase, acid phosphatase, and arylsulfatase A.

Light and electron microscopy of conjunctiva. In all four cases the conjunctiva was histologically abnormal by both light and electron microscopy.

Light microscopy. Some vacuoles and numerous dark granules that stained strongly with toluidine blue (Fig. 2) were present in both the epithelial cells and the stromal fibroblasts. The overall picture was similar in all four patients, although there were differences in extent as well as in distribution between the epithelial cells and the connective tissue components of the conjunctiva.

Electron microscopy.

Patient No. 1 (AR). Epithelial cells contained enlarged lysosomes and single-membrane limited vacuoles sometimes filled by uni- or multivesicular bodies (Fig. 3). In most of the cells, the round nucleus was still centrally localized within the cell, but in others, aggregates of the abnormal storage bodies displaced the nucleus to one side. Numerous lamellar vesicular storage bodies, probably representing abnormal lipids, were present in most of the fibroblasts (Fig. 4).

Patient No. 2 (KV). In those cells which still retained some structural integrity, small myelin deposits could be seen in the vicinity of the mitochondria and the Golgi apparatus (Fig. 5). Other cells, however,
were already filled with large lamellar and concentric inclusion bodies very similar to those which accumulate in Tay-Sachs disease (Fig. 6). The packed membranes, arranged in layers, were not always separated from the cytoplasm by a distinct membrane (Fig. 7). In some cells heterogeneity of the storage material is suggested by the variability in the periodicity of the lamellae. Pleomorphic osmiophilic material, disposed in undulating parallel sheets (Fig. 8), fills much of the cytoplasm. The same figure also shows large membrane-limited vacuoles containing finely granular material similar to that found in the mucopolysaccharidoses. Severely affected cells were grossly distended by the accumulated storage materials. The cytoplasm of some cells had the appearance of alveolar cells, with the nucleus reduced and displaced to the periphery.

**Patient No. 3 (AZ).** Giant vacuoles containing fine fibrillogranular material and limited by a single membrane occupy a
Fig. 5. Patient No. 2. Conjunctival epithelium showing abnormal deposits within the cells. Note, however, that normal cellular morphology is, on the whole, still preserved. C, Golgi apparatus; m, mitochondria; N, nucleus; MCB, membranous cytoplasmic bodies (×25,000).

Fig. 6. Patient No. 2. Conjunctival epithelial cells showing numerous lamellar and concentric bodies reminiscent of those which accumulate in Tay-Sachs disease. However, in this case they are contained within larger single membrane-bound vacuoles (×32,000).
Fig. 7. Patient No. 2. Conjunctival epithelium. Note the heterogeneity of the deposited storage material. Very few lamellar bodies are contained within vacuoles. The single membrane-bound vacuoles vary considerably in their content of fibrillogranular material. V, vacuole; MCB, membranous cytoplasmic bodies; mv, microvilli (>25,000).
large part of the epithelial cell cytoplasm (Fig. 9). These vacuoles were confluent and caused the cytoplasm to be restricted to a thin rim at the periphery of the cell. In other cells (Fig. 10), vacuoles contained structures with lamellar configuration, as well as pseudomyelinic figures. In the stromal cells, numerous membranous bodies stored within cytoplasmic vacuoles were observed.

Patient No. 4 (MS). Epithelial cells were loaded with concentric myelin-like structures (Fig. 11) which were much more prominent than the vacuolar components. In some cells, initial stages in the alteration of the nucleus were observed with a
Fig. 9. Patient No. 3. Low-power scan of conjunctiva showing extensive vacuolated epithelial cells with loss of cytoplasmic organelles. The vacuoles are coalescent and filled with clear, sparse fibrillogranular material. Arrow indicates microvilli of the superficial epithelial cells. (x6,400.)

Fig. 10. Patient No. 3. Conjunctival epithelium showing large vacuoles, empty in some areas, while other regions contain lamellar and pseudomyelinic bodies. m, mitochondria; Ep, epithelium. (x16,200.)
Fig. 11. Patient No. 4. Conjunctival epithelium showing numerous lamellar bodies filling the cytoplasm and displacing the nucleus. (×20,000.)

separation of the two layers of the nuclear envelope. The stromal fibroblasts were filled with clear vacuoles (Fig. 12).

**ERG and VEP.** These examinations were performed only on patient No. 2. A subnormal ERG was found, with both the photopic and scotopic components being affected. After seven minutes in darkness, the amplitudes of the negative and positive waves were about half of the normal values (Fig. 13). The VEP was very subnormal.

**Discussion**

Abnormalities in cellular morphology provide important information for the diagnosis and classification of many genetic disorders, particularly those caused by abnormal metabolism of lipids and/or complex carbohydrates. The three groups of inherited systemic storage diseases having frequent ocular signs include the mucopolysaccharidoses, the sphingolipidoses, and the mucolipidoses.

The specific ultrastructural changes present in the conjunctiva, as well as the absence of mucopolysacchariduria, delineates the syndrome described in this report as one of the mucolipidoses. One important feature that differentiates the mucolipidoses from the systemic mucopolysaccharidoses is that in the former group of disorders the conjunctival cells contain both single membrane-limited vacuoles filled with fibrillogranular material and lamellar bodies, while in the mucopolysaccharidoses lamellar bodies are only occasionally observed. Membranous inclusion bodies are the most characteristic neuronal cellular abnormality in the sphingolipidoses, and similar lamellar storage bodies have also been noted in conjunctival biopsies in Fabry's disease. Thus, the simultaneous accumulation of both membranous and fibrillogranular inclusion bodies, suggestive of defects in the degradation of both lipids and complex carbohydrates, is the hallmark of the ultrastructural pathology in the mucolipidoses.

The four cases described here are, from a clinical point of view, clearly different
from any other known mucolipidosis entity. Moreover, the present study has revealed that an important ultrastructural difference also exists. In mucolipidoses I, II, and III, the abnormal storage materials were found to accumulate only in the connective tissue cells of the conjunctival stroma and in those cases where it was examined, the epithelium was found to be normal. By contrast, in the present cases (as well as in some of the mucopolysaccharidoses) the conjunctival epithelium was also severely affected.

Table I lists the mucolipidoses in which corneal clouding has been described and also shows the place of ML IV among them. Other mucolipidoses in which corneal changes have not been described are listed in Table II. Present evidence suggests that the combination of moderate to severe corneal clouding at an early age, an absence of skeletal dysplasia, an absence of gargoyl-like facies, normal levels of lysosomal hydrolases, and the involvement of both the epithelial and stromal cells of the conjunctiva clearly distinguishes ML IV from the other mucolipidoses, and delineates the present cases as a distinct nosological entity. In common with the other mucolipidoses shown in Table I, psychomotor retardation is a consistent finding. However, the corneal clouding in ML IV is so prominent, and in most cases appears so early in life, that patients may be brought to the ophthalmologist sooner than to the pediatrician.

There may be some heterogeneity in this syndrome since neither the clinical nor the ultrastructural findings were identical in all of the patients. Although the ultrastructure of the conjunctiva was qualitatively similar in all four patients, there were significant differences in the severity of the cellular changes. Patient No. 2 showed the most severe ultrastructural abnormalities, with frequent cellular fragmentation; yet this child had much milder psychomotor retardation than patient No. 3, and much milder corneal clouding than patient No. 1. Thus it appears that there is little correlation between the severity of the conjunctival changes on the one hand, and either the degree of corneal clouding or the systemic manifestations on the other. Kenyon reached a similar conclusion for some other mucolipidoses. Hence, at the present time it is not clear whether the severity of these conjunctival changes is of great prognostic value.

The importance of the subnormal ERG found in patient No. 2 is also not clear.
Table I. Mucolipidoses associated with corneal clouding*

<table>
<thead>
<tr>
<th>Name of disorder</th>
<th>Corneal clouding</th>
<th>Psychomotor retardation</th>
<th>Skeletal dysplasia</th>
<th>Facial dysmorphism</th>
<th>Hepatosplenomegaly</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-gangliosidosis I</td>
<td>±</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>Metachromatic leukodystrophy, Austin variant</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
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<tr>
<td>ML I (lipomucopolysaccharidosis)</td>
<td>±</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>±</td>
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<tr>
<td>ML II (I-cell disease)</td>
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<td>+++</td>
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<tr>
<td>ML III (pseudo-Hurler polydystrophy)</td>
<td>+</td>
<td>±</td>
<td>++</td>
<td>+</td>
<td>-</td>
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<tr>
<td>ML IV</td>
<td>± or +++</td>
<td>+ or ++</td>
<td>+ or ++</td>
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</tbody>
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*Compiled from references 1, 2, 3, and 12.
- Absent.
* Mild or variable.
++ Moderate.
+++ Severe.

Table II. Mucolipidoses in which corneal clouding has not been described

- GM-gangliosidosis, Type II
- Farber's lipogranulomatosis
- Sea-blue histiocyte syndrome
- Fucosidosis
- Mannosidosis

Pigmentary chorioretinal degeneration has been described in some of the mucopolysaccharidoses, but retinitis pigmentosa has not been reported in the mucolipidoses. In ML III the ERG's have been found to be either normal or at the lower limit of normal.

There was no consanguinity between the parents of any of the four patients described here. However, all eight parents were of Jewish-Ashkenazi origin and of those antecedents who could be traced, most of them originated in Southern Poland. All of the previously described mucolipidoses appear to be inherited as autosomal recessive traits and it is reasonable to assume that ML IV is also inherited in this manner. It is interesting to note that if in fact ML IV is due to a single gene mutation that occurred in a European-Jewish community, then the location is different from that causing Tay-Sachs disease which occurred in the area of Northeast Poland. It is somewhat curious that the patients described here were all diagnosed by us in the course of one year. The question arises why a not uncommon entity such as this has not been recognized before. Conceivably there may be at least two reasons. First, a disease caused by a mutant gene in an Ashkenazi-Jewish community would appear where such a population is predominant, as in Israel. Second, the clinical signs are so subtle that these patients would have remained undiagnosed but for the recent advances in our understanding of storage diseases and the use of conjunctival biopsies to confirm the diagnosis.

The authors wish to thank Prof. E. Auerbach, Prof. G. Kohn, Dr. C. Legum, Dr. P. Nemet, Prof. A. Russell, and Dr. H. Savir for referring patients to us and for carrying out special laboratory examinations.

REFERENCES