Conjunctival ultrastructure in mucolipidosis III (pseudo-Hurler polydystrophy)

Harry A. Quigley and Morton F. Goldberg

 Conjunctival biopsies from two sibs with mucolipidosis III and from their clinically normal parents were examined by histochemistry and electron microscopy. Connective tissue cells were filled with single membrane-limited vacuoles which frequently contained membranous lamellar material. These findings indicate abnormal mucopolysaccharide and glycolipid accumulation in this disorder and suggest a mechanism for the corneal clouding in these patients.

Key words: mucolipidosis, ultrastructural pathology, conjunctiva, corneal opacification, acid mucopolysaccharide, glycolipid, mucopolysaccharidosis, sphingolipidosis

Corneal opacification is known to be associated with three large classes of gene-determined metabolic disorders (Table I). In the first group, defects of acid mucopolysaccharide (AMP) metabolism which have clinically evident corneal clouding are systemic mucopolysaccharidoses I, IV, V, and VI, as well as macular corneal dystrophy (Groenouw's type II), and an AMP disorder localized to the cornea. A second group, the sphingolipidoses, includes Fabry's disease with its characteristic whorled corneal opacities. The third group, the mucolipidoses, are inherited metabolic diseases characterized by abnormal accumulation of AMP, sphingolipids, and/or glycolipids. Among these, Ga-gangliosidosis and mucolipidosis III, (formerly pseudo-Hurler polydystrophy or pseudo-polypolydystrophy) both regularly cause corneal clouding.

Mucolipidosis III (MLS III) is a syndrome of fine homogeneous corneal opacification, coarse facies, stiff joints, short stature, mild gibbus with anterior vertebral body defects, and knock knees. In contrast to some of the disorders listed above, neither hepatosplenomegaly (and no histologic liver abnormalities), severe psychomotor retardation, excessive urine AMP, lymphocyte vacuolation, nor white blood cell granulation is present. Previous laboratory studies have demonstrated vacuolated bone marrow cells and Alcian blue-positive deposits in cultured skin fibroblasts, the latter indicating excessive AMP. The hereditary nature of MLS III is confirmed by the presence of toluidine blue metachromasia.
Table I. Differential features of metabolic diseases associated with corneal opacity

<table>
<thead>
<tr>
<th>Disease groups</th>
<th>Corneal opacity</th>
<th>Abnormal storage material</th>
<th>Skeletal dysplasia</th>
<th>Gargoyle habitus</th>
<th>Excess urine AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucopolysaccharidoses</td>
<td>MPS I (Hurler)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPS II (Hunter)</td>
<td></td>
<td>-</td>
<td>AMP and some glycolipid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPS III (Sanfilippo)</td>
<td>MPS IV (Morquió)</td>
<td>+</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPS V (Scheie)</td>
<td></td>
<td>+</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPS VI (Maroteaux-Lamy)</td>
<td></td>
<td>+</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macular corneal dystrophy</td>
<td></td>
<td>+</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucolipidoses</td>
<td>MLS I (lipomucopolysaccharidosis)</td>
<td>-†</td>
<td>AMP, glycolipid, and/or sphingolipid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLS II (I-cell disease)</td>
<td></td>
<td>-</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLS III (pseudo-Hurler polydystrophy)</td>
<td>+</td>
<td>-</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gangliosidosis</td>
<td></td>
<td>-</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannosidosis</td>
<td></td>
<td>-</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile sulfatidosis</td>
<td></td>
<td>-</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sea-blue histiocyte syndrome</td>
<td></td>
<td>-</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farber’s disease</td>
<td></td>
<td>-</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingolipidoses</td>
<td>Tay-Sachs</td>
<td>-</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niemann-Pick</td>
<td></td>
<td>-</td>
<td>AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metachromatic leukodystrophy</td>
<td></td>
<td>-†</td>
<td>Sphingolipid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fabry’s disease</td>
<td></td>
<td>-</td>
<td>Sphingolipid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Krabbe’s disease</td>
<td></td>
<td>-</td>
<td>Sphingolipid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactosyl ceramidosis</td>
<td></td>
<td>-</td>
<td>Sphingolipid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandhoff’s disease</td>
<td></td>
<td>-</td>
<td>Sphingolipid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MPS = mucopolysaccharidosis; MLS = mucolipidosis; AMP = acid mucopolysaccharide.

*Opacity seen with slit lamp in older patients.
†Opacity in one of six reported cases.
‡Questionable opacity in one case.

in skin fibroblasts from both parents of some affected sib pairs. Although there has been no previous evidence for abnormal glycolipid storage in MLS III, Spranger and Weidemann, have classified it as a mucolipidosis because it could be differentiated from the mucopolysaccharidoses due to lack of excess urine AMP.

This report summarizes the ultrastructural examinations of conjunctival biopsies from a brother and sister with MLS III and from their clinically normal parents. Conjunctiva is an excellent tissue for such study, because it is representative of connective tissue and because it is structurally similar and close to cornea.

Case reports

Patient No. 1 (JHH 133 70 61), a Caucasian boy was born August 19, 1955 to nonconsanguineous parents. Pregnancy and delivery were uncomplicated. He and his only sib (Patient No. 2) have been previously reported. Developmental landmarks were normal and his general health was good until age 7 years, when a limp, knee pain, easy fatiguability, and hyperopia requiring correction were noticed. Physical examination (1969) revealed an intelligent boy of proportionate short stature with generalized stiff joints and facies suggestive of a mucopolysaccharidosis. The chest had an increased anteroposterior diameter, and a barely audible diastolic cardiac murmur was heard. There was no enlargement of the abdominal organs. X-ray studies demonstrated the following defects: deep posterior cranial fossa; moderate platyspondyly; wide spinal disc spaces with deficiency of anterior portions of T-10 and T-12; mild gibbus with apex of T-11; flared iliac wings; poorly mineralized femoral heads and moderate coxa valga; irregularity of all long bone metaphyses; mildly flattened epiphyses; wide tubular bones and hypoplastic fifth finger phalans; and pointed proximal ends of the metacarpals. There was no excess urine AMP. No metachromatic white blood cell inclusions were seen.
Fig. 1. Light micrograph of conjunctiva, showing epithelium (E), subjacent stroma which is unusually cellular, and several vacuolated connective tissue cells (arrow) (Patient No. 2). (Epoxy-embedding, phase contrast, X200.)

Corrected vision was 20/20 bilaterally. On external examination, the corneas were clinically clouded. Slit lamp examination showed a fine, feathery haze of the corneal stroma, which was greatest in its posterior and peripheral portions. The media and fundi were normal, as was an electroretinogram.

Patient No. 2 (JHH 134 05 82), a Caucasian girl, the sister of Patient No. 1, was born October 20, 1956. Pregnancy and delivery were normal. Her health has been good, and, like her brother, she is an honor student. Physical examination (1969) revealed facies similar to her brother's and proportionate short stature. A mild systolic ejection cardiac murmur was heard in the aortic area. There was no abdominal organ enlargement. All joints, though not enlarged, had decreased range of motion. This limitation was less marked than that of Patient No. 1. A skeletal x-ray survey revealed defects similar to, but less severe than, those of Patient No. 1. There was neither excessive urine AMP nor metachromatic white blood cell inclusions.

Ocular examination was normal except for hyperopia and faint corneal haziness similar to that in Patient No. 1. An electroretinogram was normal.

Patient Nos. 3 and 4 are the clinically normal parents of patient Nos. 1 and 2.

Materials and methods

Bulbar conjunctival biopsies were taken approximately 1 cm. from the limbus and were fixed immediately in five per cent glutaraldehyde in 0.4M phosphate buffer, pH 7.3, at 25° C. for seven days. They were then washed in buffer and postfixed in two per cent osmium tetroxide in the same buffer for 30 minutes at 0° C. Tissues were embedded in epoxy resin. One-micron thick sections were cut on a Porter-Blum MT-2 microtome and were stained with methylene blue and toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and examined with a JEM 100-B electron microscope. Conjunctiva from normal control subjects and from patients with AMP metabolic defects were identically prepared for comparison.

Results

Light microscopy. Epoxy-embedded sections from the affected sibs showed connective tissue cells with abnormal vacuolation (Fig. 1). Such cells—fibroblasts and histiocytes—were more prominent in tissue from Patient No. 2. The vacuolated cells in both Patient Nos. 1 and 2 stained meta-
chromatically with toluidine blue. Tissue from both parents was indistinguishable from normal.

**Electron microscopy.**

*Patient Nos. 1 and 2.* Ultrastructural examination revealed fibroblasts and histiocytes full of single membrane-limited vacuoles which were 0.1 to 1.0 μ in diameter (Figs. 2, 3, and 4). Such vacuolation was more severe in Patient No. 2, but was qualitatively identical in both. The vacuolar matrix consisted of finely fibrillogranular material and frequent membranous lamellar inclusions (Fig. 5). These membranous lamellae were arranged both concentrically and parallel in the same cell, with lamellar width of 25 A and periodicity of 50 A* (Fig. 6). When present, such lamellar material rarely filled an entire vacuole, the remainder containing the more lucent fibrillogranular material (Fig. 6). Vacuoles were seen to be closely associated with the Golgi complex (Fig. 7), and their membranes never had attached ribosomes. Other cellular organelles were normal in appearance, but were infrequent in heavily vacuolated cells. In such cells, vacuoles seemed to coalesce into an alveolar pattern, with swelling and distortion of normal cellular shape (Figs. 2 and 3). Occasional extracellular vacuoles were noted (Fig. 3), but similar extracellular material was found in the stroma of normal conjunctiva. There were no abnormalities of conjunctival epithelium or of other connective tissue elements.

*Patient Nos. 3 and 4.* No ultrastructural abnormalities could be detected in tissue from either parent: Specifically, no fibroblast vacuolation was present (Fig. 8).

**Discussion**

The histochemical and ultrastructural findings in our two patients are the first evidence that MLS III is characterized by abnormal storage of both AMP and glycolipid. Firstly, the Alcian blue–positive staining of the skin fibroblasts, previously reported, and the toluidine blue metachromasia of conjunctival cells in this report are consistent with the abnormal accumulation of AMP. The fibrillogranular vacuoles in conjunctival cells are very similar ultrastructurally to those seen in the systemic mucopolysaccharidoses. The severity of conjunctival fibroblast vacuolation in MLS III is less than that of mucopolysaccharidosis type I (Hurler), comparable to that of types III, V, and VI (Sanfilippo, Scheie, and Maroteaux-Lamy) and greater than that in types II and IV (Hunter and Morquio). Secondly, although membranous lamellar vacuoles are present in central and peripheral nervous tissue cells in the mucopolysaccharidoses, they are infrequently seen in vacuolated conjunctival cells in these disorders. We find that membranous lamellar vacuoles are much more common in MLS III conjunctival cells. In both neural and connective tissues from the sphingolipidoses, however, most abnormal vacuoles are filled with membranous lamellar material. Thus, MLS III appears intermediate between the mucopolysaccharidoses and the sphingolipidoses in the frequency of membranous lamellar vacuoles in connective tissue cells.

The implications of this finding depend upon both the origin and the chemical nature of the membranous lamellar material. Its origin is undoubtedly intracellular for three reasons: (1) No significant amount of such material is seen outside connective tissue cells in our specimens. (2) Pinoctosis of membranous lamellar material is not observed. (3) Membranous lamellar vacuoles appear to emerge from the Golgi complex, the normal secretory pathway for intracellularly synthesized material. The only information available for chemical identification of these membranous lamellae is their comparative ultrastructural appearance. Van Mullum and Ruiter found membranous lamellae in skin fibroblasts of Fabry's disease to have a periodicity of 52 A. Weingeist and Blodi reported a membranous lamellar periodicity of 40-50 A.

---

*The periodicity is the combined width of one dense lamella and one adjacent clear space.*
Fig. 2. Electron micrograph of conjunctival stroma, with extracellular collagen (C) and several fibroblasts with labelled nuclei (N) and cytoplasm swollen by vacuoles (arrows). (Patient No. 2) (×6500.)
Fig. 3. Fibroblast processes with fibrillogranular vacuoles (v) in an alveolar pattern and some membranous lamellar vacuoles with parallel and concentric orientation (arrows). Occasional apparently extracellular vacuoles (E) are present (Patient No. 2). (×10,000.)
Fig. 4. Fibroblast nucleus (N), fibrillogranular vacuoles (v), and membranous lamellar vacuoles (arrows). Inset, from different cell, shows membranous lamellar material (mlb) and the single limiting membrane of its vacuole (arrow) (Patient No. 1). (x13,500; inset, x21,000.)
Fig. 5. Fibroblast cytoplasm illustrating the frequency and electron density of membranous lamellar vacuoles (Patient No. 2). (x86,500.)
Fig. 6. Individual membranous lamellar vacuoles at high power showing concentric (A) and parallel (B, C, D) orientation. (Patient No. 2) (A, ×143,500, B, ×123,500, C and D, ×140,000.)
Fig. 7. Two examples of membranous lamellar vacuoles (star) in close association with Golgi complexes (G). Mitochondria (M) and rough endoplasmic reticulum (E) are normal. (Top, Patient No. 2, ×56,500; Bottom, Patient No. 1, ×53,500.)
Fig. 8. Normal conjunctival fibroblasts of parents, showing nuclei (N), narrow rim of cytoplasm with rough endoplasmic reticulum (arrows), fine cellular processes, and normal lipid-containing body (L) unrelated to membranous lamellar material. (A, Patient No. 3, x13,000; B, Patient No. 4, x14,500.)
in ocular connective tissue cells of a Fabry's disease carrier. Terry and Korey\(^{17}\) found lamellae with a periodicity of 50 to 60 Å in cells from patients with Tay-Sachs disease, which on biochemical analysis were half ganglioside with some cerebroside and cholesterol. Ultrastructurally identical membranous lamellae were produced in vitro\(^{19}\) with a mixture of the above compounds. All of these measurements are similar to the 50 Å periodicity of lamellae observed in MLS III. However, such spatial arrangements are subject to variation in temperature, degree of hydration, and fixation, in addition to the molecular composition of the lamellae.\(^{17}\) Thus, it is difficult to determine the precise chemical nature of membranous lamellar material with the use of dimensions derived from electron microscopic observations alone. We conclude only that, based on their ultrastructural similarity to the storage material seen in sphingolipid disorders, the membranous lamellar material of MLS III is composed of intracellularly produced glycolipid which is stored abnormally along with excessive AMP. This evidence therefore supports Spranger's and Weidemann's contention\(^{5}\) that MLS III is a mucolipidosis.

Reports of connective tissue pathology in other MLS's contain somewhat similar findings. Liver cells from both MLS I\(^{20}\) and fucosidosis\(^{21}\) have membranous lamellar vacuoles. In MLS II (I-cell disease),\(^{22}\) connective tissue cells of conjunctiva, skin, and liver have a significant percentage of membranous lamellar vacuoles. Likewise, fibroblasts in the cornea, sclera, and ciliary body of GM\(_1\)-gangliosidosis patients\(^{6}\) have infrequent membranous lamellar inclusions, although retinal ganglion cells are full of them. There is histochemical evidence for accumulation of abnormal amounts of AMP in both MLS II and GM\(_1\)-gangliosidosis. In addition, the degree of vacuolation in connective tissue cells from these two disorders is more severe than that in MLS III, as would be expected from their more devastating clinical courses.

The absence of light or electron microscopic abnormalities in the parents of our patients was somewhat unexpected, as both parents of two other sib pairs with MLS III have had cultured skin fibroblasts which have shown toluidine blue metachromasia.\(^{7}\) If, as seems likely, this disorder is inherited as an autosomal recessive trait, our findings suggest that its morphologic expression in the conjunctival cells of two obligatory heterozygotes is beyond the resolution of these histologic and ultrastructural techniques.

Finally, in view of the similarity between conjunctival pathology in MLS III and the mucopolysaccharidoses, the mechanism of corneal clouding may very well be identical; namely, accumulation of abnormal storage material in and around stromal keratocytes.\(^{11}\)

The authors wish to thank John E. Dowling, Ph.D., for providing facilities, and Charles I. Scott, M.D., for making clinical data available.

REFERENCES


