Mucolipidosis II (I-cell disease): Ultrastructural observations of conjunctiva and skin

Kenneth R. Kenyon and Judith A. Sensenbrenner

Conjunctival and skin biopsies from a 25-month-old patient with mucolipidosis II (I-cell disease) were studied by electron microscopy. In both tissues the subepithelial connective tissue was markedly hypercellular, as histiocytes and fibroblasts showed extensive vacuolation by single membrane-limited inclusions with fibrillogranular and membranous lamellar contents. The Schwann cells and axonal processes of peripheral nerves and the vascular perithelial cells were similarly affected. Histochemical and ultrastructural evidence indicates that these storage vacuoles may be derived from lysosomes which contain excessive amounts of acid mucopolysaccharide and glycolipid. Biomicroscopically visible granularity of the corneal stroma suggests the accumulation of these storage substances also within the cornea. Symmetrically enlarged corneas were also present.

Key words: I-cell disease, mucolipidosis, mucopolysaccharidosis, sphingolipidosis, acid mucopolysaccharide, glycolipid, sphingolipid, megalocornea, corneal clouding, ultrastructure, conjunctiva and skin, lysosome, fibrillogranular vacuole, membranous lamellar vacuole

The mucolipidoses (MLS's) are a newly categorized group of inherited metabolic storage diseases which seemingly occupy an intermediate position between the mucopolysaccharidoses and the sphingolipidoses. The ten disorders currently included in this group are listed in Table I.

Clinically, these disorders share many features of the mucopolysaccharidoses (MPS's), including coarse facial features, skeletal dysplasia, and mental retardation (Table II). Biochemically, however, the MLS’s resemble the sphingolipidoses (SLS’s) with respect to normal urinary excretion of acid mucopolysaccharide (AMP) and visceral storage of glycolipid and/or sphingolipid in addition to AMP. The significant ocular manifestations of the MLS’s are also common to both the mucopolysaccharide and sphingolipid disorders. These ocular features are variable and include corneal clouding (in generalized
Fig. 1. Light photomicrograph of the conjunctiva shows connective tissue cells with vacuolated cytoplasm (asterisks) to be most numerous in the collagenous stroma (C) which underlies the conjunctival epithelium (E). (Paraphenylenediamine, phase contrast, x800.)

Table I. Classification of the mucopolysaccharidoses (MPS's), mucolipidoses (MLS's), and sphingolipidoses

<table>
<thead>
<tr>
<th>Mucopolysaccharidoses</th>
<th>Mucolipidoses</th>
<th>Sphingolipidoses</th>
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<tr>
<td>MPS I (Hurler)</td>
<td>Generalized gangliosidosis</td>
<td>Tay-Sachs disease (Gm-gangliosidosis I)</td>
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<td>MPS II (Hunter)</td>
<td>(Gm-gangliosidosis I and II)</td>
<td>Sandhoff's disease (Gm-gangliosidosis II)</td>
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<tr>
<td>MPS III (Sanfilippo)</td>
<td>Fucosidosis</td>
<td>Niemann-Pick disease</td>
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<td>MPS IV (Morquio)</td>
<td>Mannosidosis</td>
<td>Gucher's disease</td>
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<tr>
<td>MPS V (Scheie)</td>
<td>Juvenile sulfatidosis, Austin type</td>
<td>Fabry's disease</td>
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<tr>
<td>MPS VI (Maroteaux-Lamy)</td>
<td>MLS I (lipomucopolysaccharidosis)</td>
<td>Krabbe's disease</td>
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<td></td>
<td>MLS II (1-cell disease)</td>
<td>Infantile metachromatic leukodystrophy</td>
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<td></td>
<td>MLS III (pseudo-Hurler polydystrophy)</td>
<td>Lactosyl ceramidosis</td>
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<td></td>
<td>Farber's disease</td>
<td>(chronic Niemann-Pick disease)</td>
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<td></td>
<td>Sea-blue histiocytie syndrome</td>
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gangliosidosis, MLS I, and MLS III) and macular cherry-red spot (in generalized gangliosidosis, MLS I, and Farber's disease).

MLS II, the I-cell variant, is a rare disorder which was first described in 1967 by Leroy and DeMars, who found coarse cytoplasmic inclusions in cultured fibroblasts (which they designated I-cells or inclusion cells) from two children with this disease. At present, only about ten cases have been reported. On the basis of these reports, MLS II may be characterized as an autosomal recessive trait which is present at birth and is associated with severe psychomotor retardation, early cessation of growth, Hurler-like facies with characteristic gingival hyperplasia, and extreme skeletal dysplasia. Additional clinical signs include mild hepatic enlargement,
Fig. 2. Light photomicrograph of the skin reveals many vacuolated histiocytes (asterisks) in the superficial dermis. E, epidermal cells; C, collagenous dermis. (Paraphenylenediamine, phase contrast, x1,000.)

Table II. Comparative features of the mucopolysaccharidoses, mucolipidoses, and sphingolipidoses (after Spranger and Wiedemann)

<table>
<thead>
<tr>
<th>Mucopolysaccharidoses</th>
<th>Mucolipidoses</th>
<th>Sphingolipidoses</th>
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<tbody>
<tr>
<td>Gargoyle-like facies</td>
<td>Gargoyle-like facies</td>
<td>No gargoyle-like facies</td>
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<tr>
<td>Skeletal dysplasia</td>
<td>Skeletal dysplasia</td>
<td>No skeletal dysplasia</td>
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<tr>
<td>Mental retardation (variable)</td>
<td>Mental retardation (variable)</td>
<td>Normal mental retardation</td>
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<tr>
<td>Abnormal mucopolysacchariduria</td>
<td>Normal mucopolysacchariduria</td>
<td>Normal mucopolysacchariduria</td>
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<tr>
<td>Visceral storage of AMP</td>
<td>Visceral storage of AMP and glycolipids</td>
<td>Visceral storage of glycolipids</td>
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<tr>
<td>Ocular features (variable)</td>
<td>Ocular features (variable)</td>
<td>Ocular features (variable)</td>
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<tr>
<td>Corneal clouding</td>
<td>Corneal clouding</td>
<td>Corneal clouding</td>
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<tr>
<td>Retinal pigmentary degeneration</td>
<td>Macular cherry-red spot</td>
<td>Macular cherry-red spot</td>
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<td>Optic atrophy</td>
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restricted joint mobility, and unusually tight skin. Corneal opacities have been described in only one case of probable MLS II, and macular cherry-red spot has never been reported.

Laboratory findings include vacuolated lymphocytes and bone marrow cells and normal urinary excretion of AMP. The refractile cytoplasmic inclusions in cultured fibroblasts are periodic acid–Schiff (PAS) and Sudan black positive and, following treatment with chloroform-methanol, are metachromatic with toluidine blue thereby indicating storage of both glycolipids and AMP.

The pathogenesis of MLS II is as yet unclear. Biochemical studies of I-cell tissues and cultured cells are not in complete agreement; however, decreased activity of beta galactosidase is the most consistent finding to date.

To our knowledge, the histopathology of MLS II has not been previously reported. Thus, the present ultrastructural study of
Fig. 3. In the superficial stroma of the conjunctiva, histiocytes and fibroblasts are numerous. The cytoplasm of these cells shows an alveolar reticulated appearance, as abnormal storage material (asterisks) has distended the cells, displaced their nuclei (N), and seemingly excluded other cytoplasmic organelles. E, epithelium; C, collagenous connective tissue. (x5,400.)

conjunctiva and skin from a patient with I-cell disease is presumably the initial histopathologic description of this disorder.

Case report

The patient (JHH 140 25 75) was the 25-month-old son of unrelated Caucasian parents. Following an unremarkable pregnancy and delivery, coarse facies and inguinal hernias were noted, and subsequent psychomotor development was extremely slow. In November, 1970, physical examination revealed an alert but otherwise severely retarded child with the facial and skeletal stigmata of I-cell disease. The liver was enlarged to 8 cm. below the right costal margin and a spleen tip was palpable. Everywhere on his body, the skin was smooth, tight, and thickened.

Ophthalmic evaluation disclosed that the child followed objects and fixated well in all fields of gaze. There was neither strabismus nor nystagmus. The pupils were round, regular, and reactive to light. External examination was normal except for an increased corneal diameter of 12.5 mm. bilaterally. The corneas appeared grossly clear but showed diffuse fine stromal granularity by slit-lamp examination. Intraocular pressures were 13.0 mm. Hg (Schiotz) bilaterally on two occasions.
Dilated pupil funduscopy was entirely within normal limits; specifically, the disc and vessels appeared normal, and neither macular cherry-red spot nor retinal pigmentary change was evident. Laboratory studies showed metachromatically vacuolated lymphocytes, normal bone marrow, and normal mucopolysacchariduria. Skeletal x-rays showed severe dysostosis multiplex.

**Materials and methods**

Biopsies of inferior bulbar conjunctiva and anterior abdominal skin were obtained under general anesthesia and immediately processed for histochmical study and electron microscopy.

For histochemistry, frozen unfixed sections of skin were stained with toluidine blue, Alcian blue, PAS, oil red O, Sudan black, aldehyde fuchsin, acid phosphatase, glucuronidase, and ribonuclease.

For electron microscopy, conjunctival and skin specimens were fixed in two per cent osmium tetroxide (buffered with 0.14M veronal acetate, pH 7.3, 280 milliosmolar) for 20 minutes at 0°C. and for one hour at room temperature. These tissues were then dehydrated in graded alcohols, embedded in Araldite epoxy resin, and sectioned on a Porter-Blum MT-2 ultramicrotome. For orientation and light photomicrography, thick sections (~ 1 μ) were stained with paraphenylene-diamine. Thin sections were stained with uranyl acetate and lead citrate and were examined with a JEM 100-B electron microscope.

**Results**

Histologic examination of the conjunctiva revealed the accumulation of large mononuclear histiocytes with foamy cytoplasm in the superficial stroma (Fig. 1). Similar cells in the deeper conjunctival connective tissue were also distended by fine cytoplasmic vacuoles. In the superficial dermis of the skin, cellular vacuolation was equally impressive (Fig. 2).

Histochemical studies of the skin demonstrated connective-tissue cells which stained positively for acid mucopolysaccharide by the Alcian blue technique. These cells, however, did not show toluidine blue metachromasia (although chloroform-methanol extraction was not attempted) and did not stain with PAS, lipid stains, or lysosomal enzyme stains.

By electron microscopy, numerous ballooned histiocytes were especially striking in the superficial stroma of the conjunctiva.

Fig. 4. At higher magnification, the cytoplasm of a conjunctival histiocyte (area indicated by large asterisk in Fig. 3) is filled by an alveolar reticulum of unit membranes (each ~ 60 Å in thickness; arrows) interspersed with fibrillogranular material (asterisk) and membranous material arranged as concentric (white circle) or parallel (black circle) lamellae. (×83,500.)
The cytoplasm of these cells was packed with abnormal material which often excluded all other organelles except the nucleus. Discrete intracellular vacuoles were seldom evident; rather, rupture and coalescence of vacuoles had apparently produced an alveolar reticulum of membranous material. At higher magnification, the storage material was seen to be composed of sparse, fine, granular substance and of membranous inclusions arranged as parallel or concentric lamellae (Fig. 4). Frequently, these membranous lamellar inclusions assumed elaborate myelin-like configurations in which a characteristic periodicity of 50 to 60 Å could be resolved (Fig. 5). In the deeper conjunctival stroma, connective tissue cells were also extensively involved (Fig. 6), but there the abnormal storage inclusions retained their integrity as discrete, single membrane-limited vacuoles which contained predominantly fibrillar granular material (Fig. 7). In these cells, membranous lamellar vacuoles, lipoid
globules, and polymorphous electron-dense inclusions were less prominent than in the subepithelial connective tissue cells.

In the skin, histiocytes and fibroblasts of the superficial dermis displayed vacuolation which was identical to that of the conjunctival connective tissue cells (Fig. 8).

In both conjunctiva and skin, the Schwann cells and axonal processes of peripheral nerves showed these same vacuolar changes (Figs. 9 and 10). The epithelial cells of the conjunctiva and the epidermis were seemingly unaffected. The vascular endothelium of the lymphatics and capillaries was also not involved, but perithelial cells of the capillaries consistently contained prominent storage vacuoles (Fig. 11).

In both tissues, neither extracellular vacuoles nor abnormal accumulations of extracellular material were discernible by electron microscopy.

Discussion

In our patient with MLS II and in previous studies of the MLS's, the findings
suggest that both AMP and glycolipids are the abnormally accumulated substances in these storage diseases.

Histochemical evidence for combined storage of AMP and glycolipid in MLS II is afforded by the aforementioned work of Leroy and associates and of Matalon and co-workers. Both groups found positive staining with PAS and Sudan black and metachromasia with toluidine blue in cultured I-cells, which also showed an increased content of AMP and glycolipid by direct biochemical assay. These findings are entirely consistent with observations of liver tissue from the present patient in which hepatic reticuloendothelial cells held Sudan black-positive material, which by electron microscopy proved to be large lipoid or membranous lamellar inclusions. In generalized gangliosidosis, visceral histiocytes have also been shown to contain storage material which was PAS positive, weakly sudanophilic, and weakly toluidine blue metachromatic, whereas in the ocular tissues affected by this disorder, vacuolated histiocytes demonstrated an increased AMP content by the colloidal iron reaction. Similarly, in mucolipidosis III (pseudo-Hurler polydystrophy), Alcian blue staining of skin fibroblasts and toluidine blue metachromasia of conjunctival connective tissue cells have also indicated an abnormal accumulation of AMP.

In the MPS's, wherein AMP storage predominates, electron microscopy reveals storage vacuoles with predominantly fibrillogranular material.
Fig. 8. In the skin, histiocytes of the superficial dermis show extensive vacuolation by single membrane-limited vacuoles which contain fibrillogranular material and membranous lamellae (asterisks). Inset, Two membranous lamellar vacuoles (extreme right of figure) are shown at higher magnification. E, epithelial cell; BM, basement membrane of epidermis; C, collagenous connective tissue; N, nucleus. (×8,750; inset, ×40,000.)

logranular contents (see Discussion, ref. 13), whereas in the SLS's, membranous lamellar vacuoles have been shown to contain glycolipid (see Discussion, refs. 12 and 14). Ultrastructural studies of a variety of tissues affected by MLS's have consistently disclosed large numbers of both fibrillogranular and membranous lamellar vacuoles. And for MLS II, in particular, the cellular defect appears to be virtually a composite picture of the MPS's and SLS's. Thus, the simultaneous occurrence of both vacuolar types in the MLS's constitutes strong morphologic evidence for the accumulation of both AMP and glycolipid in these disorders and additionally attests to their intermediate position between the MPS's and the SLS's.

The concept of lysosomal diseases, first introduced in 1964 by Van Hoof and Hers, has subsequently been applied to those metabolic disorders (including the MPS's and SLS's) in which storage substances are thought to accumulate within lysosomes, the impaired or deficient hydrolytic enzymes of which render these substances indigestible. Reports of significantly decreased activity of one or more of the acid hydrolases in tissues of MLS II patients are consistent with the possibility of a lysosomal disorder in this disease. Additional histochemical studies of cultured I-cells have also revealed increased activity of the lysosomal enzyme, acid phosphatase, an observation which suggests that the I-cell inclusions may be activated lysosomes. This finding was also especially striking in the liver biopsy specimen of the present patient, wherein focal epitheloid cell granulomas showed markedly increased histochemical staining for acid phosphatase, which by electron microscopy proved to
correspond to innumerable intracellular vacuoles with fibrillogranular and membranous lamellar contents. Furthermore, in MLS I and in various MPS's and SLS's, ultrastructural localization of acid phosphatase activity has been specifically associated with these storage vacuoles. On the basis of such histochemical and electron microscopic studies, therefore, it would appear that in the MLS's, as well as in the MPS's and SLS's, the storage vacuoles may be derived from altered lysosomes which have become engorged with accumulated AMP and glycolipid substances.

Among the clinical features of the present patient, symmetrically enlarged corneas and fine granularity of the corneal stroma are the only findings of direct ophthalmic interest. Both megalocornea and buphthalmos have been described in association with the MPS's. In the absence of signs of increased intraocular pressure, therefore, the enlarged corneas of our patient may be a connective tissue abnormality which is related to defective AMP metabolism. Corneal clouding, which is associated with many of the metabolic storage diseases, presumably results from the light-scattering effect of the abnormally accumulated storage substances which perturb the lamellar organization of the corneal stroma (see Discussion, refs. 14 and 22). Accordingly, several ultrastructural studies of corneas affected by various MPS's and by generalized gangliosidosis have demonstrated excessive intracellular and extracellular AMP in quantities which correlated with the extent of corneal clouding. In MLS II, therefore, it is probable that AMP and glycolipid deposits throughout
the cornea account for the fine stromal granularity observable by slit lamp in the present patient.

It is of additional interest, however, that in many of these storage disorders, corneal changes seem to occur independently of pathologic alterations in adjacent tissues. In particular, we have studied conjunctival ultrastructure in several of the MPS's and MLS's and have found that conjunctival pathology is not always a reliable indicator of corneal opacification. In the systemic MPS's, for example, although the degree of vacuolation of conjunctival connective tissue cells paralleled the extent of corneal clouding in MPS types I, II, V, and VI, this relationship did not hold for MPS's III and IV. Moreover, in macular corneal dystrophy (an inherited localized disorder of AMP metabolism) and in Goldberg's MLS variation, the cornea is clouded (presumably by AMP and glycolipid accumulation) even though the conjunctiva shows essentially no cellular defect. And finally, in comparing MLS II (I-cell disease) and MLS III (pseudo-Hurler polydystrophy), we have determined that the ultrastructural alteration of the conjunctiva is far more severe in the former disorder, whereas corneal clouding is clinically significant only in the latter disease. The physicochemical basis for these specific reactions of the cornea to each disorder of AMP-glycolipid metabolism remains to be determined.

The authors gratefully acknowledge Dr. John E. Dowling for use of laboratory facilities, Dr. Robert G. Wyllie for histochemical studies, Mr. Kenneth A. Linberg for technical assistance, Mr. Terry George, R.B.P. for photomicrography, Mrs. Faye...
Fig. 11. The peripheral cell (P) of a dermal capillary contains many vacuoles with granular, membranous, and electron-dense contents. The endothelial cells (E) lining the capillary are apparently unaffected. N, nucleus; asterisk, capillary lumen; BM, basement membrane. (x13,500.)

Snyder for secretarial assistance, and Drs. W. R. Green, V. A. McKusick, H. A. Quigley, and C. I. Scott for critical review of the manuscript.

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