Macular corneal dystrophy: Ultrastructural pathology of corneal endothelium and Descemet's membrane

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Corneal buttons from four patients with macular corneal dystrophy (MCD) were examined histochemically and ultrastructurally with particular emphasis on the abnormalities of the endothelium and Descemet's membrane. Abnormal deposits of acid mucopolysaccharide (AMP) were histochemically demonstrable within both the endothelial cells and Descemet's membrane. By electron microscopy, these deposits corresponded to fibrillogranular material visible as membrane-bound vacuoles within the endothelial cytoplasm and as vesicular deposits throughout Descemet's membrane. The similarity of these alterations to those of the keratocytes and stroma in MCD indicates that the endothelium may be primarily involved in this disorder and may constitute the source of the extracellular AMP in Descemet's membrane. Guttate excrescences of Descemet's membrane in MCD ultrastructurally resembled those of Fuchs' combined dystrophy. Comparison of the corneal lesions in MCD and in systemic mucopolysaccharidoses suggests different biochemical lesions in these disease states.

Key words: macular corneal dystrophy, mucopolysaccharidosis, acid mucopolysaccharide, Descemet's membrane, corneal endothelium, corneal guttata, histochemistry, electron microscopy.

Macular corneal dystrophy (MCD) was first described by Groenouw in 1890, and along with granular and lattice dystrophies is recognized as one of the three classical forms of inherited corneal dystrophies which primarily involve the stroma. MCD is inherited as an autosomal recessive trait and is clinically characterized by bilateral corneal clouding resulting from a diffuse stromal haze extending to the limbus, plus irregularly shaped stromal opacities which are most numerous in the axial region. Decreasing visual acuity is often first evident by the age of ten to twelve years and is slowly progressive, usually requiring surgical restoration by keratoplasty.

The pathogenesis of MCD is currently thought to involve a disorder of acid mucopolysaccharide (AMP) metabolism which...
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Fig. 1. Light photomicrograph of posterior cornea (Case 2). Endothelial cells (E) stain intensely positive for AMP. Guttate excrescences (*) of Descemet's membrane (DM) are frequent. Histochemically demonstrable accumulations of AMP in Descemet's membrane cannot be visualized in this black and white photomicrograph. The stroma also shows intense staining AMP accumulations (arrows). (AC, anterior chamber; colloidal iron; x800.)

is seemingly confined to the cornea. By histochemical methods, it has been shown that abnormal amounts of AMP, consisting for the most part of a keratan sulfate proteoglycan, accumulate both intracellularly and extracellularly in the MCD cornea. Although Jones and Zimmerman initially advanced the theory that mucoid degeneration of stromal collagen with liberation of AMP was responsible for the pathologic findings, Klintworth and Vogel's observation of abnormal AMP storage material within intracytoplasmic vacuoles derived from dilated rough-surfaced endoplasmic reticulum pointed toward a primary metabolic disorder of the keratocyte. Cotlier's recent discovery of a deficiency of the lysosomal hydrolitic enzyme alpha-galactosidase in cornea and in cultured conjunctival fibroblasts of an MCD patient is consistent with both Klintworth and Vogel's hypothesis and with the general concept that recessively inherited disorders involve defects in enzymatic cellular processes. Thus, though further biochemical studies will be required to establish the basic metabolic defect, present evidence favors the possibility that MCD, like the systemic disorders of AMP metabolism, might be an inborn lysosomal disease.

The stromal lesions of MCD have been described by both light and electron microscopy, but the pathologic alterations of the endothelium and Descemet's membrane have received only incidental attention. To our knowledge, no detailed ultrastructural description of the posterior corneal layers in MCD has been presented, nor has it been adequately resolved whether these abnormalities are primary or are secondary to stromal changes. In this study, therefore, corneal buttons from four MCD patients were examined histochemically and ultrastructurally in an attempt to elucidate further the pathogenesis of MCD, particularly as it affects the corneal endothelium and Descemet's membrane.

Brief clinical summaries

Case 1. I. K. (JHH 84-69-14, EP 28169), a 29-year-old white male who had experienced de-
Fig. 2. Electron micrograph of corneal epithelium and Bowman's layer (Case 1). The basal epithelial cells (Ep) show nonspecific changes including interruptions of the basement membrane (BM). Bowman's layer is notable for abnormal accumulations of extracellular material (*) (presumably AMP) and histiocytes filled with vacuoles (V) containing fibrillogranular material. (×32,500.)

creasing vision bilaterally since age eleven. One brother had MCD, but the parents were normal. A clinical diagnosis of MCD was made and a lamellar keratoplasty done on the right eye in 1959; the tissue for the present study was obtained during penetrating keratoplasty of the same eye in May, 1967. A penetrating keratoplasty was done on the left eye in 1960, at which time histopathologic examination confirmed the diagnosis of MCD.

Case 2. B. L. (JHH 49-25-84, EP 29452), a 33-year-old white female with a history of decreasing vision bilaterally since the age of twelve. Her family history was negative for visual problems. A clinical diagnosis of MCD was made in 1965, and a penetrating keratoplasty performed on the right eye at that time. The diagnosis of MCD was confirmed by histopathologic examination. The present tissue is from a penetrating keratoplasty performed on the left eye in Nov., 1968.

Case 3. R. W. (JHH 134-16-22, EP 33152), a 34-year-old white male with a history of decreasing visual acuity bilaterally. Family history was negative for ocular disease. A clinical diagnosis of MCD was made, and a penetrating keratoplasty was performed on the left eye in Nov., 1970, at which time the present tissue was obtained. Histopathologic examination confirmed the diagnosis of MCD.

Case 4. M. S. (JHH 19-19-42, EP 34824), a 50-year-old black female with a history of decreasing vision bilaterally since youth. Family history included one sister with MCD. A diagnosis of MCD was made clinically in 1969, and it was histopathologically confirmed following penetrating keratoplasty of the left eye at that time. Tissue for the present study was obtained during penetrating keratoplasty of the right eye performed in Feb., 1972.

Materials and methods
Surgically excised corneal buttons were immediately fixed in 2.5 per cent glutaraldehyde (buffered with 0.067 M sodium cacodylate, pH 7.2, 365 milliosmolar) at room temperature for two to twelve hours. The buttons were then halved, and
one portion was routinely processed for light microscopy; stains included hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome, and colloidal iron with and without hyaluronidase. The other portion of the button was prepared for electron microscopy by postfixation in 2 per cent osmium tetroxide (buffered with 0.14 M veronal acetate, pH 7.2, 250 milliosmolar) for one hour at room temperature, dehydration in graded alcohols, and embedding in Araldite epoxy resin. Thin sections were cut on a Porter-Blum MT-2 ultramicrotome, mounted on uncoated copper grids, doubly stained with uranyl acetate and lead citrate, and examined on an RCA EMU-3F electron microscope at 50 kv.

Results

Light microscopy. Examination of histologic sections from the four corneal specimens revealed consistent alterations. The epithelium showed nonspecific swelling and variations in thickness. Bowman's membrane was disrupted by large mononuclear cells with foamy cytoplasm and in places was replaced by a material which had a granular, pale gray appearance with hematoxylin and eosin. The stroma demonstrated similar material in vacuolated keratocytes and extracellular deposits as described by others.1-7, 11-13 Descemet's membrane was irregular in thickness and showed guttate swellings posteriorly (Fig. 1); in areas free of guttata, the thickness of Descemet's membrane measured between 9 and 14μ. The endothelium was somewhat flattened and was occasionally interrupted by the thicker guttata.

Special histochemical stains revealed abnormal accumulations of AMP within Bowman's histiocytes, keratocytes, and endothelial cells, as well as throughout the stroma and Descemet's membrane. This material stained faintly blue with Masson's trichrome, magenta with periodic acid-Schiff, deep blue with colloidal iron, and it was insensitive to hyaluronidase. Colloidal iron is especially effective in demonstrating the striking deposits of AMP in the endothelium (Fig. 1) and faint accumulations in Descemet's membrane.

Electron microscopy. The corneal epithelium appeared ultrastructurally unre-
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markable, and no intracellular inclusions suggestive of storage material could be visualized. Occasional interruptions in the epithelial basement membrane may have been related to the presence of histiocytic cells and abnormal deposits of a granular extracellular substance in Bowman's layer (Fig. 2). The cytoplasm of these sub-epithelial histiocytes contained membrane-bound vacuoles filled with a fibrillogranular material of moderate electron density. These abnormalities of the superficial cornea, although only occasionally seen, were evenly distributed from the periphery to the center of the specimens.

In the corneal stroma, numerous membrane-limited, intracytoplasmic vacuoles filled the keratocytes (Fig. 3). Most of these vacuoles contained a fibrillogranular material of moderate electron density, but a few vacuoles consisted of membrane-like, osmiophilic whirls in an electron-lucent space. Similar fibrillogranular and membranous deposits were interspersed throughout the stromal collagen fibrils, but no obvious relationship to the keratocytes was evident. Small membranous figures seemed to predominate extracellularly, whereas intracellularly the fibrillogranular deposits were more numerous. These stromal changes were similar to those described by others, with however, dilated cisternae of rough-surfaced endoplasmic reticulum could not be unequivocally identified as the origin of the intracellular vacuoles as has been previously proposed.

The anterior zone of Descemet's membrane was ultrastructurally unremarkable as it consistently measured ~4 μm in thickness and demonstrated the usual banded appearance with ~1,100 Å periodicity (Fig. 4). The posterior zone of Descemet's membrane, in contrast, was honeycombed with deposits of abnormal material; more anteriorly, these deposits appeared as electron-lucent areas within which were small (0.1 to 0.5 μm diameter) membrane-like, osmiophilic whirls (Figs. 4 and 6), similar to those seen extracellularly in the stroma. More posteriorly (subjacent to the endothelium), the deposits were fibrillogranular and comparable ultrastructurally to the abnormal material found in Bowman's layer, stroma, and endothelium, although no limiting membrane could be seen (Figs. 4 and 6). Faint banding of ~1,100 to 1,200 Å periodicity was also evident throughout the posterior zone.

The guttate excrescences seen by light microscopy appeared by electron microscopy as localized thickenings of the posterior zone of Descemet's membrane, consisting of fibrillogranular deposits interspersed with banded figures of ~1,100 to 1,200 Å periodicity (Fig. 6). Fissures within these guttata were seemingly filled by ingrowths of degenerated endothelial cytoplasm. Except in the guttate areas, Descemet's membrane was not unusually thickened, averaging ~13 μm. No cells were found in Descemet's membrane, and the observed ultrastructural changes were consistent throughout all specimens.

The endothelial cells contained numerous membrane-bound, intracytoplasmic vacuoles containing fibrillogranular material similar to that evident elsewhere in the cornea, although no osmiophilic figures were seen (Fig. 5). Occasional dilated cisternae of rough-surfaced endoplasmic reticulum contained a granular material, but no direct connections between the endoplasmic reticulum...
ulium and the vacuoles were unequivocally demonstrable. No connection was apparent between the storage material within the endothelium and the extracellular deposits in Descemet's membrane. Occasional discontinuities of the endothelium were probably artifact.

Discussion

The abnormalities of corneal endothelium and Descemet's membrane in MCD have been thought to be secondary changes representing diffused or phagocytized material from the stroma. Our observations, however, are more consistent with the hypothesis that the endothelium is primarily involved in MCD, and that it contributes the deposits in Descemet's membrane. The endothelial accumulations are histochemically proven to be AMP and are ultrastructurally identical to the fibrillogranular vacuoles of the stromal keratocytes. Given the appearance of the intervening Descemet's membrane, these endothelial vacuoles do not seem to be the result of diffusion from the stromal AMP sources; nor are they likely to be phagocytized material, as there was no ultrastructural evidence of phagocytosis or pinocytosis. The role of the endoplasmic reticulum in the pathogenesis of the endothelial storage vacuoles cannot be excluded, although no direct connec-
tions were seen. Such a role has been suggested for the dilated endoplasmic reticulum in the keratocyte, but we were unable to confirm this finding. This difficulty may reflect our limited ultrastructural survey of the stroma, or it may be a manifestation of the advanced state of our cases.

Descemet's membrane is thought to be secreted by the endothelium as its true basement membrane. The zonal appearance of Descemet's membrane in MCD as disclosed by electron microscopy suggests that the abnormal AMP deposits may have been derived from the endothelium and laid down simultaneously with the basement membrane material of Descemet's membrane. The osmiophilic membranous figures seen more anteriorly in the posterior zone may represent an older, degenerated form of the AMP-containing vacuole as has been speculated for similar figures in the stroma, or, less likely, they may be a different kind of storage material which was produced during an earlier period of life.

The initial 3 to 4 μ thick portion of Descemet's membrane is produced during the fetal period. In MCD, the anterior zone of Descemet's membrane most certainly seems to correspond to this fetal portion of the membrane. The normal ultrastructure of this anterior zone may reflect the fact that MCD is not a congenitally apparent disease. Marked abnormalities of the postnatally secreted portions of Descemet's membrane might, therefore, indicate basic differences between fetal and adult endothelial cells. If, for example, the deposits in Descemet's membrane are the result of the gradually progressive overflow of AMP from an abnormal endothelium, then it may be speculated that the endothelial cell is not sufficiently compromised in fetal life. Another possibility is that abnormal amounts of AMP are not produced by fetal endothelial cells in MCD, thereby implying developmental differences in endothelial metabolism or perhaps maternal factors which may correct the metabolic defect.

In addition to MCD, posterior guttata excrescences of Descemet's membrane have been described in association with other disorders of the corneal endothelium, including Fuchs' combined dystrophy, posterior polymorphous degeneration, Hassall-Henle wart formation, and interstitial keratopathy. The ultrastructural appearance of guttata in MCD most closely resembles that of Fuchs' combined dystrophy, with bulging and thickening of Descemet's membrane posteriorly to over twice normal, fissures containing ingrown degenerate cellular processes, and banded figures of ~1,100-1,200 Å periodicity. Thus, it would appear that the formation of excrescences in Descemet's membrane may be a nonspecific response of an irritated or dystrophic endothelium. For MCD, in particular, the injurious effects of AMP deposition in the endothelial cells may cause their fibroblastic metaplasia with resultant formation of excess basement membrane and collagen, as has been proposed in Fuchs' combined dystrophy.

Histochemical and ultrastructural demonstrations of intracellular AMP accumulation in keratocytes and endothelium with apparent sparing of the epithelium would seem to indicate that MCD is a metabolic disorder which is manifestly limited to cells originating from embryonic mesothelium. In the systemic mucopolysaccharidoses, in contrast, evidence of AMP in corneal epithelial cells as well as in keratocytes and endothelial cells points toward involvement of both ectodermally and mesodermally derived cells. Moreover, extracellular AMP deposits throughout the stroma and Descemet's membrane are especially prominent in MCD, whereas in systemic mucopolysaccaridosis corneas, stromal AMP accumulations are seldom evident and abnormalities of Descemet's membrane have never been reported. Finally, in systemic mucopolysaccharidoses, AMP storage vacuoles seem to be derived from the Golgi complex, whereas current concepts of the source of AMP in MCD have implicated the endoplasmic reticulum with no detectable abnormalities of the Golgi com-
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plex. Thus, these differences in the embryologic origin of the involved cell types, in the distribution of abnormal storage material, and in the apparent intracytoplasmic sources of the accumulated AMP lend support to the theory of different biochemical lesions in the AMP metabolism of MCD and systemic mucopolysaccharidoses.25

REFERENCES


Fig. 6. Electron micrograph of posterior excrescence of Descemet's membrane (Case 2). The posterior zone of Descemet's membrane is thickened posteriorly by excretion material displaying banded configurations (circled) and fissures (*). Abnormal granular and membranous deposits are also evident in the posterior zone of Descemet's membrane, while the anterior zone appears unaffected (see also Figs. 4 and 5). A portion of an endothelial cell is seen to contain a fibrillar granular vacuole (V). Inset: At higher magnification, an exoscent area shows banded figures (circled) and a fissure (*) containing degenerate endothelial cytoplasm. (S, stroma; AC, anterior chamber; ×10,200; Inset: ×27,600.)