Abnormalities in Spinal Neurons and Dorsal Root Ganglion Cells in Tangier Disease Presenting with a Syringomyelia-like Syndrome

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Abstract. A woman with homozygous Tangier disease had progressive syringomyelia-like neuropathy. She died with cardiac failure at age 61. A sural nerve biopsy taken at age 60 had shown lipid storage in Schwann and interstitial cells, and a pronounced loss of unmyelinated fibers. The neurons of the L5 spinal ganglion and, to a lesser extent, all neurons of the sacral spinal cord, contained large lipid inclusions which in electron micrographs differed from those in Schwann and satellite cells. There was no storage material in glial cells. The neuronal inclusions were membrane-bound and consisted of electron-dense and electron-lucent components. There was evidence of neuronal death in the spinal ganglion, and a diameter histogram showed that small cytons had preferentially been lost. The inclusions probably were secondary lysosomes or residual bodies, and resembled giant lipofuscin granules. Nevertheless, they were uncolored and displayed weak autofluorescence as compared to the aging pigment in control ganglia. It is tentatively suggested that the syringomyelia-like neuropathy in Tangier disease represents a lysosomal storage disorder preferentially affecting small dorsal root ganglion cells.

Key Words: High-density-lipoprotein deficiency; Motoneurons; Neuronal storage disease; Syringomyelia-like syndrome; Spinal ganglia; Tangier disease.

INTRODUCTION

Tangier disease is a rare familial disorder characterized by a near absence of high-density-lipoprotein (HDL) cholesterol, and cholesterol and cholesteryl ester storage in the histiocytes of various tissues (1, 2). With the patient on whose study this report is based a total of 35 cases have been reported. The patients may be at increased risk for premature vascular disease (3). A neurogenic disorder was demonstrated in 21 patients. The neuropathy may occur as a relapsing multiple mononeuropathy, or as a syringomyelia-like syndrome with dissociated sensory loss and faciobrachial muscle weakness and wasting (4–9) (seven cases, including the present one). Sural nerve biopsy, in both forms, shows that Schwann cells and interstitial cells contain large lipid droplets which typically appear lucent in electron micrographs. These droplets are not enclosed by a limiting membrane. The mononeuropathic form is characterized by demyelination and remyelination, whereas in the syringomyelia-like syndrome axonal degeneration of small myelinated and unmyelinated fibers prevails (2, 4–7). The cause of axonal degeneration is unknown, and studies of nerve cells are not available (2). An autopsy was performed in only one patient who died at the age of six years. Large collections of cholesterol-laden macrophages were found in numerous tissues, but not in the central nervous system. The brain investigated

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in standard sections appeared normal. Whether the spinal cord or spinal ganglia were studied is not stated (10).

We believe that this report for the first time describes the light and electron microscopic findings in spinal ganglion and anterior horn cells of a patient with Tangier disease and a syringomyelia-like syndrome.

CASE REPORT

This female patient had had bilateral facial palsy since the age of 40 years. Over the followings years, a loss of temperature and pain sensation occurred; she developed weakness of the tongue, atrophy of the intrinsic hand muscles, and patchy anhidrosis. The diagnosis, based on clinical and electrophysiological findings, was syringomyelia. At age 55, she complained of burning pain of the palate so that she could not use her artificial denture; at the same time anosmia was found. At age 60, an aortic valve replacement became necessary. At that time, the serum cholesterol level was at the lower limit of normal (2.2–3.3 mM; normal 3.5–8.2 mM), the triglyceride level was two times normal, and the HDL cholesterol level was less than 5% of the lower limit of normal. Further clinical and laboratory findings were in agreement with a typical case of homozygous Tangier disease (1).

The in vivo kinetics of cholesteryl ester within the aortic wall were assessed in connection with the operation (11). The rate of cholesteryl ester hydrolysis in the aortic wall was lower than in control patients which were studied during valve replacement operations as well. These results suggested that, in Tangier disease, impaired hydrolysis might contribute to the intracellular accumulation of cholesteryl esters. A detailed biochemical description of this case is in preparation.

A sural nerve biopsy taken at age 60 showed typical lipid deposits in Schwann cells, modest axonal degeneration of myelinated fibers, a pronounced deficit of unmyelinated fibers, and large axonal sprouts without myelin sheaths. There were no onion bulbs. The light microscopical investigation of the nerve biopsy (Dr. Kamienecka) revealed modest loss of both large and small fibers (deficit 24%); de- and re-myelination were seen in teased fibers.

The patient died at age 61 with cardiac failure.

MATERIALS AND METHODS

At autopsy, 72 hours after death, an L5 spinal ganglion and a short segment of the sacral spinal cord were fixed in 2.5% glutaraldehyde–0.3% acrolein in Ringer solution. The L5 spinal ganglia taken from two males aged 55 and 65 years who had died from a malignant tumor and coronary infarction, respectively, served as controls. Some of the material was postfixied in osmium tetroxide and embedded in epoxy resin (Embed 812; Electron Microscopy Sciences, Ft. Washington, PA, USA); the rest was postfixied in 4% formalin and was embedded in methacylate (Technovit 710, Kulzer, Germany). Sections 2–5 μm thick were cut with glass knives. The epoxy sections were stained with p-phenylenediamine, and selected areas of the blocks were cut for electron microscopy. The methacylate sections were stained with acidic cresyl violet. Unstained methacylate sections 10 μm thick of the spinal ganglion were tested for autofluorescence of lipofuscin (12, 13); blue excitation and 500-, 530-, and 590-nm barrier filters were used.

The size of the cytonus of the spinal ganglia was determined on micrographs of 5-μm sections enlarged 400 times. At the level of the nucleoli, the perimeters were measured with the aid of a digitizer tablet (MOP 2, Kontron, Germany), and were divided by 3.14 to obtain “idealized” diameters which are independent of the irregular configuration of the cells. A correction formula for the different size of the nucleoli of large and small neurons was not used.

RESULTS

Light Microscopy

*Spinal Cord:* Cross-sections viewed at low magnification appeared normal. At the level studied, there were no cavities and no abnormal accumulations of glial cells. There were no signs of neuronal death or neuronophagia. High magnification light microscopy showed normal Nissl bodies, but the perikarya and proximal dendrites in addition contained granular masses which were unstained with cresyl violet, and appeared dark after osmication (Fig. 1). The same type of deposit was found in interneurons and also in dorsal horn neurons. No abnormal deposits were detected in glial cells or around blood vessels. The neuronal deposits when unstained did not show the yellow tint characteristic of lipofuscin; in sections stained with p-phenylenediamine and viewed with phase optics the masses were distinctly black and did not show the luminosity of lipofuscin.

*Spinal Ganglion:* Numerous large but conspicuously few small ganglion cells were present. There was no evidence for an irregular distribution of large and small cytons (Fig. 2A). A histogram of ganglion cell diameters revealed a shift of the cyton diameters to the right as compared to histograms of normal subjects (Fig. 3). In methacrylate sections many cells appeared "foamy" (Fig. 2B–G). The Nissl substance of all neurons was dispersed; this might, however, be due to the delay in fixation. Lipofuscin appearing brown in unstained methacrylate sections was seen in histiocytes but not in neurons. Satellite cells and Schwann cells contained large vacuoles which were not osmiophilic. Epoxy sections viewed with phase optics regularly revealed whorls of satellite cells marking the site of vanished neurons (Fig. 4). Nageotte nodules were scarce in methacrylate sections.

The ganglia of the control subjects showed large and small ganglion cells which contained clusters of lipofuscin granules which usually were concentrated in the periphery of the cell. The overall density of cytons appeared considerably higher than in the patient. Vacuolar inclusions as shown in Figure 2 were not found. Also in these ganglia, interstitial cells were filled with lipofuscin granules.

Lipofuscin contained in ganglion cells and interstitial cells of the control ganglia showed strong autofluorescence. The sections from the patient gave a relatively weak response, and autofluorescence seemed to be restricted to the inclusions of the interstitial cells. Nevertheless, the neuronal inclusions exhibited autofluorescence, in particular in incident light, but it was much weaker than in the control ganglia.

Electron Microscopy

Most neurons of the spinal cord and almost all dorsal root ganglion cells contained an abundance of inclusions which distinctly differed from the “empty” vacuoles in satellite and Schwann cells. The neuronal inclusions measured 1–3 μm in diameter and consisted of a granular highly osmiophilic substance which was intermingled with rounded bodies of low electron density measuring 0.1–2.0 μm in diameter. The inclusions were surrounded by a distinct limiting membrane. Periodic structures were not found (Figs. 1D, 5).

The “empty” vacuoles in Schwann and satellite cells morphologically corresponded to the putative lipid inclusions which have been described in all reports of nerve biopsies in Tangier disease (2) and which were present in the patient’s nerve biopsy as well.

Several leukocytes were encountered; they did not contain stored material.

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Fig. 1. Anterior horn cells. A. A granular highly osmiophilic mass occupies a large part of the perikaryon. Epoxy embedding, p-phenylenediamine, bright field. B, C. Unstained deposits. Note distinct Nissl bodies. Methacrylate embedding, cresyl violet. D. Electron micrograph showing the neuronal inclusions. Bars: 100 μm (A–C) and 1 μm (D).

It was not possible to evaluate neuropil or nerve fibers because autolytic changes were pronounced.

The spinal ganglion cells of the control subjects contained numerous lipofuscin granules which were smaller and less tightly packed than the neuronal inclusions,
Fig. 2. Dorsal root ganglion cells (L5). Methacrylate embedding, cresyl violet. A. Ganglion cells are scarce, and mainly large cells are present. In places unstained deposits are visible. B–G. Collection of neurons to show vacuolar inclusions. The large vacuoles (G) may develop from smaller granules (C). Bars: 0.5 mm (A) and 100 μm (B–G).
Fig. 3. Histograms of diameters of perikarya of the L5 dorsal root ganglion from the patient (below), and from two normal subjects. Small neurons are lacking in the patient (n: number of perikarya measured).

and the relative amount of the osmiophilic component was much higher than in the neuronal inclusions of the patient (Fig. 6).

Interstitial cells which presumably were histiocytes contained large clumps of osmiophilic material, both in the patient and in the control subjects. This material was not enclosed by a distinct membrane, and there were rarely electron-lucent vacuolar components.

DISCUSSION

These observations described indicate that Tangier disease may involve neuronal storage of membrane-bound inclusions which probably contain lipids (Figs. 1, 5). The shift of the histogram of cyton diameters (Fig. 3) suggests loss of small spinal ganglion cells; the storage of cytoplasmic inclusions might have caused additional
enlargement of the perikarya. The size distribution in the control patients was comparable to that in the normal material published by Dyck et al (14). A selective loss of small ganglion cells correlates with the loss of pain and temperature sensation in the patient, and the deficit of unmyelinated fibers in the sural nerve biopsy. Anhidrosis might have been due to the concomitant affection of sympathetic ganglia. Whether the motor deficits in tongue and hands were due to loss of anterior horn cells or to peripheral demyelination (6) remains obscure.

The inclusions in numerous perikarya of the spinal ganglion had replaced much of the cytoplasm (Fig. 2), and might have caused neuronal death. Nevertheless, there exists a discrepancy between the pronounced and apparently generalized affection of the nerve cells, and the relatively mild and slowly progressing neurogenic disorder. Epoxy-embedded sections studied with phase contrast revealed many whorls of satellite cells marking the sites of lost neurons. This was less well seen in methacrylate sections in which only the nuclei of the satellite cells were stained. The scarcity of
Fig. 5. Dorsal root ganglion cell (L5). Electron micrographs. A. Lower-power micrograph to show size and packing density of the inclusions. B. Neuronal inclusions consisting of an electron-dense fine-granular substance and electron-lucent vacuoles. The inclusions are surrounded by a distinct membrane. Bar: 1 μm.
typical Nageotte nodules (clusters of satellite cell nuclei) may be due to the large size of the lipid-filled satellite cells, but it may also be due to the histological technique. Shrinkage appeared less pronounced than after paraffin embedding, and the section thickness was only one fifth to one third of that of routine paraffin sections.

The neuronal inclusions in several cases resembled lipofuscin granules (Figs. 1, 5, 6), but they were larger than the "wear-and-tear" pigment normally deposited in neurons of aged subjects, and the amount of the non-osmiophilic vacuolar component was higher. The appearance of the neuronal inclusions in electron micrographs suggests that they consist of at least two lipid components which differ with respect to solubility. The more soluble component would have been extracted during the embedding procedure, and would have given rise to the electron-lucent vacuoles within the inclusion. Lipid extraction probably accounts for the "empty" vacuoles in Schwann and satellite cells as well. The neuronal inclusions are membrane-bound and it is conceivable that they are secondary lysosomes. The specimens had been thoroughly fixed, and it was not possible to test whether the inclusions contained lysosomal enzymes.

The hypothesis that the neuronal inclusions are secondary lysosomes, or residual bodies of lysosomes that have lost enzyme activity, is compatible with their classification as atypical lipofuscin granules. Lipofuscin granules are derived from lysosomes and contain the undigestible remnants of cellular metabolism, mostly lipids. In young animals, neuronal lipofuscin granules are homogeneously osmiophilic, but later peripheral vacuoles occur which are due to the incorporation of more soluble lipids (15). In Tangier disease, soluble lipids may be stored in excess. This would explain the large size of the inclusions, their vacuolization in electron micrographs.
(Figs. 1, 5), and the foamy appearance of the cells in light micrographs (Fig. 2). Autofluorescence of the neuronal inclusions in the Tangier disease patient was weak as compared to lipofuscin granules in the controls. By contrast, the lipofuscin granules of the interstitial cells showed strong fluorescence, both in the controls and in the patient. The autofluorescence of lipofuscin persists after embedding involving exposure to strong solvents (12), and is probably associated with the dense component of the granules. The excess of soluble lipids in Tangier disease (Fig. 5A) may reduce autofluorescence. It is also possible that the composition of the dense substance in Tangier disease is abnormal. The lipopigment of the stellate ganglion of young and aged normal subjects differs with respect to color, ultrastructure, and autofluorescence; adjacent ganglion cells of aged subjects may contain different types of lipopigment (16). The authors conclude that residual bodies accumulate different cellular degradation products and thus different mixtures of fluorophores (16).

In vivo studies with labeled cholesterol in the present patient suggested that cholesteryl ester hydrolysis was reduced in the aortic wall (11). Cholesteryl esters may accumulate in lysosomes. Schmitz et al (17) exposed cholesteryl ester-laden macrophages of mice to HDL and report that HDL is bound by specific cellular receptors, internalized, and, after interaction with the margin of lipid droplets, it is resecreted. Cultured human monocytes of control subjects bound and internalized HDL as well, transported it through the cytoplasmic compartment, and resecreted it without significant degradation. The binding of normal HDL to cultured monocytes from three patients with Tangier disease was moderately increased, but, instead of being resecreted, the bulk of HDL was stored in secondary lysosomes (18). The authors suggest that cellular “trapping” of HDL and ineffective cholesterol clearance may explain the macrophage cholesteryl ester storage in Tangier disease. Whether this abnormality of the intracellular membrane traffic in Tangier monocytes relates to the neuronal lipid storage is unknown. If this were the case, it would be justifiable to classify the neuropathy in Tangier disease as lysosomal storage disease. The characteristic ultrastructural appearance of the lysosomal inclusions in established lipid storage disorders of the nervous system probably reflects the physicochemical properties of the stored lipids, rather than the fact that the origin of these inclusions is basically different from that of “normal” residual bodies or lipofuscin. It is conceivable that neuronal storage of lipid occurs in the form with a syringomyelia-like neuropathy only. In fact, the question has been raised whether the clinical heterogeneity of the neurogenic symptoms in Tangier disease reflects different metabolic errors (6).

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