Progressive Nerve Lesion in a Disease-Arrested Leprosy Patient. An Electron Microscopic Study[1]

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Leprosy is characterized by peripheral nerve lesions and the pathologic changes have been studied and described by many workers using the light microscope ([4, 7, 11, 12, 14, 18, 20, 25, 27]) and also the electron microscope ([1, 5, 13, 15–17, 19, 21, 22]). The mechanism of destruction of nerves in different types of active leprosy has been described earlier. In lepromatous leprosy *M. leprae* are present in perineurial cells, Schwann cells, intraneural macrophages and endothelial cells. Multiplication of *M. leprae* within these cells is often followed by necrosis. A gradual degeneration and destruction of Schwann cells is mainly responsible for nerve fiber loss in lepromatous nerve lesions ([17]). In tuberculoid leprosy, sensitized Schwann cells may be surrounded and infiltrated by immunologically activated macrophages and be destroyed ([15]). Essentially, destruction of Schwann cells due to the presence of *M. leprae* or its products is responsible for nerve paralysis in active patients of both lepromatous and tuberculoid leprosy.

Occasionally patients having different types of leprosy are seen to develop paralysis long after they have become inactive and disease arrested, even while antileprosy treatment is being continued. The paralysis seems to be insiduous, gradual and progressive. The pathologic changes in nerve lesions in such patients are not well understood. In this report, the electron microscopic appearance of an ulnar nerve from one such patient is recorded and the possible mechanism of nerve destruction in it is discussed.

MATERIALS AND METHODS

Case history. A 27 year old male reported to his physician complaining of burning sensation of both hands and feet for three years. He had no skin lesions suggestive of leprosy but had developed obvious ulnar and median nerve paralysis of his left hand. He was diagnosed as having leprosy of the purely neural type and was given treatment with diaminodiphenylsulfone (DDS), 100 mg daily for the next three years. During the last six months of this period his medical attendant noticed the patient developing ulnar and median paralysis of his right hand also and referred him to us.

On examination, the patient was a 30 year old male with complete ulnar and median nerve paralysis of both hands. The ulnar and the median nerve of both upper extremities were moderately thickened and were hard. No skin lesions were present. The skin smears studied from four standard sites, namely earlobe, cheek, chin and buttocks, were negative and his lepromin reaction was Mitsuda positive. He was diagnosed as a patient with resolving borderline tuberculoid leprosy of the purely neural variety.

The right ulnar nerve, which showed signs of paralysis during the last six months of the three year treatment period, was biopsied for light and electron microscopic study. The lepromin nodule, measuring over 5 mm in diameter, was also biopsied for histopathologic examination. The lepromin biopsy was fixed in formol-Zenker solution for four hours and was transferred to 70% alcohol. After 24 hours it was processed and paraffin sections were made for H & E and acid-fast stains. The nerve biopsy was divided into two pieces. One piece was immediately cut further into many small bits 3 mm long and 1 mm thick, fixed in 2% glutaraldehyde solution at 4°C, and processed for electron microscopic study. One micron sections were made and stained according to the method described by Estable-Puig *et al* ([8]). Ultrathin sections were made and stained with lead citrate and urynal acetate. The other piece was fixed in formol-Zenker solution, processed for paraffin sections and H & E and acid-fast stains were done.

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RESULTS

Lepromin biopsy. The lepromin nodule showed a small collection of epithelioid cells and lymphocytes infiltrating the dermal collagen. Acid-fast staining showed a few bacilli. It was consistent with a moderately positive lepromin reaction as seen in a borderline tuberculoid patient.

Histopathology of the nerve. The H & E section of the nerve showed marked thickening of the perineurium. The architecture of the nerve was destroyed and replaced almost entirely by bundles of hyalinized fibrous tissue in which several spindle-shaped cells could be seen. A few scattered round cells, consisting mainly of lymphocytes, were seen and no macrophages were identified. Myelinated axons, although seen, were very rare. Acid-fast staining showed no organisms.

The 1 μ section stained by the technic described by Estable-Puig *et al* ([8]) showed thickened perineurium. The rarity of myelinated axons observed in the H & E sections was confirmed. There was hardly any inflammatory reaction except for the presence of an occasional lymphocyte.

Electron microscopy. The electron microscopic observations confirmed the light microscopic appearance. There was complete loss of normal nerve architecture, which was replaced by small bundles of tissue made up of collagen fibrils and fine Schwann cell processes containing many nonmyelinated axons (Fig. 1). The myelinated nerve fibers were few and their Schwann cells showed marked atrophy and an electron dense cytoplasm (Fig. 2). No active demyelination was seen. Onion bulbs composed of collagen fibrils, Schwann cell processes and axons were seen (Figs. 3, 4). A few nonmyelinated fibers showed complete necrosis (Fig. 5). Although parts of the basement membrane surrounding the necrotic axons were identified, there was no Schwann cytoplasm.

Occasionally Schwann cells contained large membrane-bound vacuoles with degenerating fragments of *M. leprae* (Fig. 6). In one, the bacterial plasma membrane could be well identified inside a phagolysosome (Fig. 7). A few bizarre cells containing large vacuoles of varying sizes and scattered dense mitochondrial bodies were seen (Fig. 8). These cells did not have much cytoplasm and were surrounded by fibrillar material which were obviously bundles of collagen.

The perineurium showed marked proliferation of perineurial cells forming several layers of attenuated processes (Fig. 9). There was also a significant increase in collagen fibrils of the perineurium. Some of the perineurial cells contained many dense bodies, and others showed vacuoles with light staining amorphous material.

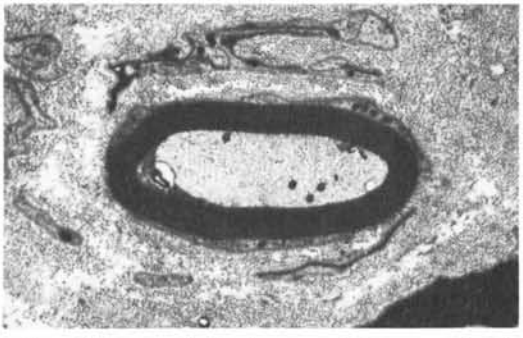

FIG. 2. A myelinated axon within a markedly atrophied Schwann cell. The cytoplasm of the Schwann cell is electron dense. X12,000.

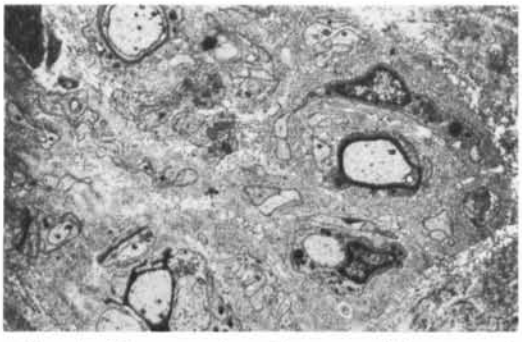

FIG. 1. The normal architecture of the nerve is replaced by bundles of collagen, irregularly arranged Schwann cell processes and axons. X 7,000.

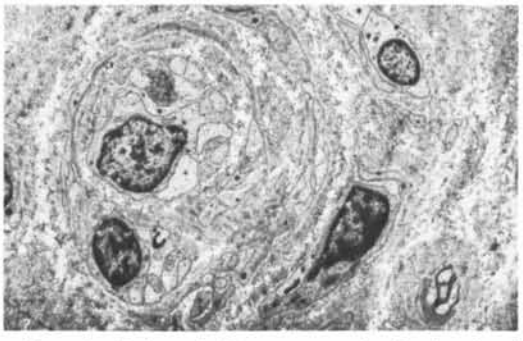

FIG. 3. Onion bulb composed of whorls of Schwann cell processes containing axons and intermingled with collagen fibrils. X7,000.

DISCUSSION

The interesting features in this leprosy patient were that he had no obvious skin lesions or bacilli in skin smears but had multiple nerve lesions and a positive lepromin test. The histopathology of nerve and lepromin biopsy was diagnostic of purely neural borderline tuberculoid leprosy. However, the unusual feature that is considered in this paper is that he developed a progressive lesion of his right ulnar and median nerves even after 2.5 years of regular antileprosy treatment with DDS when ordinarily one would expect healing and regression of existing nerve lesions.

Histopathologic examination showed

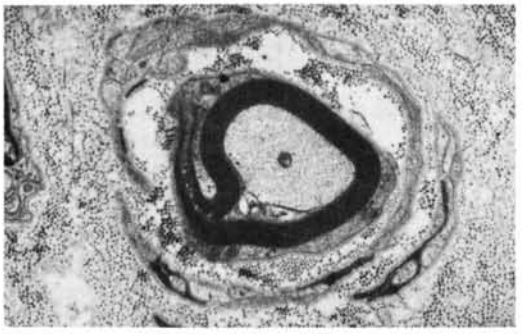

FIG. 4. Onion bulb with a myelinated axon at its center. X 15,000.

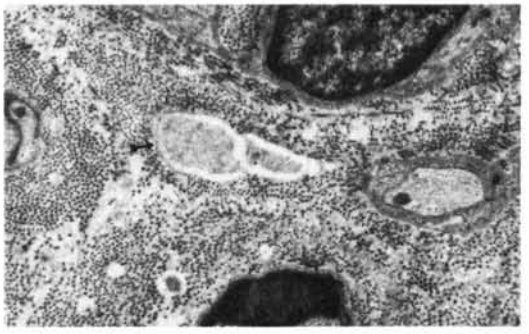

FIG. 5. A nonmyelinated axon undergoing necrosis (arrow). Part of the basement membrane of its Schwann cell is still visible. X 20,000.

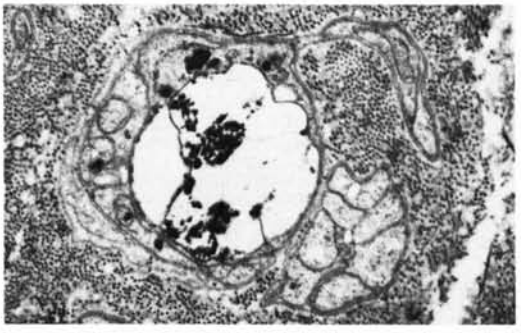

FIG. 6. Degenerating fragments of *M. leprae* in a large phagosome within a Schwann cell. X 20,000.

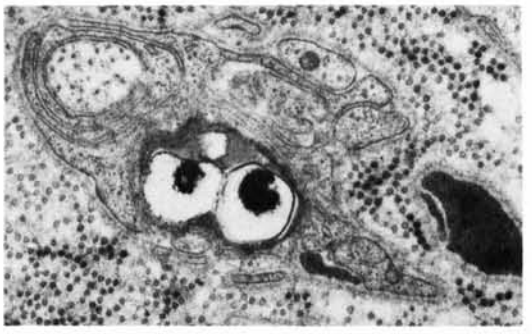

FIG. 7. Cross sections of two clearly identifiable *M. leprae* within a phagolysosome in a Schwann cell. X 60,000.

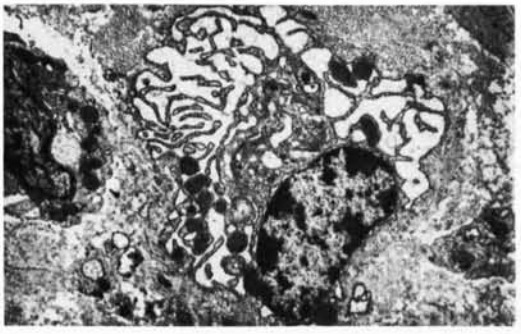

FIG. 8. Bizarre fibroblast with markedly dilated endoplasmic reticulum. X 12,000.

FIG. 9. Perineurial cell processes showing gross atrophy. The perineurium is infiltrated with bundles of collagen. X 7,000.

hardly any inflammatory reaction. There were no acid-fast bacilli but the nerve tissue was replaced by fibrous tissue as expected in any healed leprosy lesion. This histopathologic appearance is almost the end stage of a leprosy lesion in a nerve.

Electron microscopic study of this lesion was most productive. It confirmed the light microscopic picture of destruction of nerve architecture and fibrous replacement of nerve tissue. In addition, several fragmented *M. leprae* were demonstrated inside Schwann cells. It is known that it takes many months, if not years, before the intraneural bacilli are killed and eliminated during antileprosy therapy. In our experience we have seen acid-fast bacilli in Schwann cells even after 16 years of DDS therapy. Therefore, for a patient with borderline and lepromatous leprosy, prolonged or even lifetime therapy is advocated to prevent relapse of the disease.

In lepromatous leprosy it has been shown that *M. leprae* proliferate in Schwann cells ([17]). Finlayson *et al* ([10]) suggested that the response of Schwann cells in borderline leprosy is also similar and that a great majority of bacilli in the nerve were found within Schwann cells. Although intra-axonal bacilli have been demonstrated by some workers ([1, 19, 30]), it is a rare finding and its role in the destruction of nerve parenchyma may not be of any significance. In this patient *M. leprae* were scarce, were not even seen in histopathologic sections, and were identified within Schwann cells (Figs. 6, 7) only during electron microscopic examination after prolonged search. He also had had continuous antileprosy treatment for 2.5 years, therefore, there is no doubt that the proliferation of bacilli was not the cause of destruction of nerve tissue.

It has been shown that hypertrophic neuropathy can be produced by introducing crystals internally into the nerve ([29]). It is quite possible that fragments of degenerating and dead *M. leprae* can serve as crystal-like particles to produce a hypertrophic neuropathy. Further, the considerable reactive proliferation of collagen fibrils may cause ischemic necrosis of axons as seen in this patient. Carayon *et al* ([3]) have shown ischemic changes in leprosy nerves.

Onion bulb formation was frequently seen in this study. It is not a specific lesion and can be found in many chronic neuropathies ([2, 6, 9, 24, 26, 28]). The whorled processes were distinctly Schwann cell processes. Additionally, collagen fibrils formed the major part of the onion bulb. Segmental demyelination and remyelination is thought to be the underlying pathologic lesion in hypertrophic neuropathies producing onion bulbs. Therefore, it is reasonable to suggest that in leprosy also the main pathologic process is segmental demyelination.

Another interesting feature in this study was the finding of a few globoid shaped, abnormal cells containing large vacuoles and surrounded by collagen fibrils. These cells are likely to be bizarre fibroblasts as described by Schoene *et al* ([23]) in the nerves of patients with herditary sensory neuropathy, and the vacuoles seen in the cytoplasm of the cells could be abnormally-dilated endoplasmic reticulum as suggested by these authors. These abnormal endoneural fibroblasts are not unique for hereditary sensory neuropathies, and can also be seen in acquired lesions such as leprosy.

SUMMARY

An ulnar nerve biopsy from a patient with purely neural leprosy of the borderline tuberculoid group, who developed ulnar and median paralysis after 2.5 years of DDS therapy, was examined using light and electron microscopes. The nerve parenchyma was largely replaced by collagen fibrils. There were many onion bulbs similar to those seen in hypertrophic neuropathies. Bizarre fibroblasts such as those seen in hereditary sensory neuropathy were also demonstrated. A few Schwann cells contained *M. leprae*.

It is suggested that considerable proliferation of fibrous tissue may be a reactive phenomenon in response to the continued presence of fragmented *M. leprae* and their products. Ischemia following a marked progressive increase of intraneural collagen is an important cause of atrophy of Schwann cells followed by segmental demyelination and necrosis of the axons in this healed leprosy patient.

RESUMEN

Empleando microscopías electrónica y de luz, se examinó una biópsia del nervio ulnar de un paciente con lepra puramente neural del grupo intermedio-tuberculoide quien desarrolló parálisis ulnar y mediana después de 2.5 años de

tratamiento con DDS. El parénquima del nervio estuvo reemplazado de manera muy importante por fibrillas colágenas. Se observaron muchas "cabezas de cebolla" similares a las encontradas en neuropatías hipertróficas. También se observaron fibroblástos de apariencia caprichosa como los encontrados en los casos de neuropatías sensoriales hereditarias. Algunas células de Schwann contuvieron *M. leprae.*

Se sugiere que la considerable proliferación del tejido fibroso puede ser el resultado de una reacción inducida por la continua presencia de bacilos de la lepra fragmentados y sus productos. La isquemia que sigue al marcado y progresivo incremento de la colágena intraneural es causa importante de la atrófia de las células de Schwann, la cual, es seguida por desmielinización segmental y necrósis de los axones.

RÉSUMÉ

On a examiné, par microscopie ordinaire et microscopie électronique, une biopsie du nerf cubital provenant d'un malade atteint de lèpre neurale pure du type tuberculoïde borderline. Ce malade avait developpé une paralysie du nerf cubital et du nerf médian après 2, 5 années de traitement par la DDS. Le parenchyme nerveux était dans l'ensemble remplacé par des fibrilles collagènes. De nombreux bulbes d'oignons étaient présents, semblables à ceux que l'on voit dans les neuropathies hypertrophiques. Des fibroblastes étranges tels que ceux l'on voit dans les neuropathies sensorielles héréditaires ont été également mis en évidence. Quelques cellules de Schwann contenaient *M. leprae.*

On suggère qu'une prolifération considérable du tissu fibreux peut constituer un phénomène réactionnel en réponse à la présence continuelle de fragments de *M. leprae* et de leurs dérivés. L'ischémie qui suit une augmentation graduelle notable du collagène intraneural, constitue une cause importante d'atrophie des cellules de Schwann, qui a été suivie par une démyélinisation segmentaire et par la nécrose des axones chez ce malade guérit de la lèpre.

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