

Axonal Spherical Bodies in the Peripheral Nerves of Leprosy Patients¹

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ABSTRACT

Spherical bodies, roughly 10 μm in diameter, which have not been reported before, were found in the peripheral nerve axons of specimens collected during post-mortem examination of leprosy patients.

These bodies were found in the fascicles of all peripheral nerves of the extremities examined (median, radial, ulnar, peroneal and sciatic nerves). Their incidence was not related to the type of leprosy. The area immediately below the thickened perineurium, a feature associated with leprosy, often showed a large number of spherical bodies.

When observed under a transmission electron microscope, the spherical lesions often showed a lamellar structure, although some of them were amorphous. No structure resembling organelles was seen within the bodies.

Observation with the merge technique showed a clearly lamellar structure in most of the spherical bodies. These bodies and the surrounding myelin sheaths were partially polarized.

The axonal spherical bodies observed in our study seem to represent lesions gradually formed due to glycoprotein denaturation over long periods of time and to be associated with leprosy-caused thickening of the perineurium of peripheral nerves.

RÉSUMÉ

Des corps sphériques, mesurant environ 10 μm de diamètre, qui n'ont pas encore été rapportés, furent trouvés dans les axones des nerfs périphériques prélevés à l'autopsie de patients lépreux.

Ces corps furent retrouvés dans les faisceaux de tous les nerfs périphériques des extrémités examinées (nerfs médian, radial, ulnaire, péroné et sciatique). Leur incidence n'était pas liée au type de lèpre. La zone immédiatement en dessous d'un périméurium épaissi, un caractère associé à la lèpre, était fréquemment riche de ces corps sphériques.

Lorsque observés au microscope électronique à transmission, ces corps sphériques montraient fréquemment une structure lamellaire, bien que certains étaient amorphes. Aucune structure ressemblant à une organelle ne fut décelée dans ces corps.

L'observation par une technique de concaténation a révélé une structure clairement lamellaire dans la grande majorité des corps sphériques. Ces corps et les manchons myéliniques environnants n'étaient que partiellement polarisés.

Ces corps sphériques des axones, observés dans notre étude, semblent représenter des lésions progressives à long terme de dénaturation des glycoprotéines et être associés aux épaississements du périméurium des nerfs périphériques causés par la lèpre.

RESUMEN

Se observaron cuerpos esféricos de aproximadamente 10 μm de diámetro en los axones de especímenes de nervios periféricos colectados durante el examen post-mortem de pacientes con lepra. Estos cuerpos esféricos, que no se habían descrito antes, se encontraron en los fascículos de todos los nervios de las extremidades examinados incluyendo los nervios mediano, radial, ulnar, peronal y ciático. Su incidencia no estuvo relacionada con el tipo de

¹Received for publication on 22 July 2003. Accepted for publication on 14 January 2004.

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lepra y se observaron con más frecuencia por debajo del perineurio engrosado característico de la lepra.

Observados al microscopio electrónico de transmisión los cuerpos esféricos mostraron una estructura laminar, aunque algunos de ellos aparecieron amorfos. No se observaron estructuras sugerentes de organelos dentro de los cuerpos esféricos. Además de su estructura laminar, estos cuerpos y las capas envolventes de mielina aparecieron parcialmente polarizados.

Los cuerpos esféricos axonales observados en nuestro estudio parecen representar lesiones formadas gradualmente por la desnaturalización de glicoproteínas a lo largo de periodos prolongados de tiempo y parecen estar asociados con el engrosamiento del perineurio de los nervios periféricos ocasionado por la lepra.

During our 40 years of experience of post-mortem examinations of leprosy patients, we have detected spherical bodies in the peripheral nerve axons of these patients. Many reports on peripheral nerve lesions observed in leprosy patients have been based on electron microscopy examinations. These reports have often mentioned the presence of *Mycobacterium leprae* in the axons, but none of them have reported the presence of axonal spherical bodies. For the study presented here, axonal spherical bodies were observed under light and electron microscopes at magnifications up to $\times 1000$.

We used a new method known as merge technique (¹), which allows for simultaneous viewing of an object under both a polarized microscope (PM) and a differential interference contrast microscope (DIC) within a common visual field.

MATERIALS AND METHODS

Subjects from which specimens were obtained. Peripheral nerve specimens collected from 6 cadavers during post-mortem examination at the "Oku Komyoen" National Sanatorium were used for this study. One of the specimens was relatively old (collected in 1983), but the other specimens were collected fairly recently (three in 1995 and two in 1996). The specimens were collected from 6 cases whose history follows. In all cases, leprosy-related peripheral nerve disturbance was observed.

Case 1. An 89-year-old male with lepromatous leprosy, who was diagnosed at age 17 with Hansen's disease, and was admitted to the sanatorium when he was 27. At age 89, he was hospitalized because of mild motor paralysis of the extremities. During his hospitalization, he developed fever and increased sputum, leading to death from dyspnea in 1983.

Case 2. A 76-year-old female with tuberculoid leprosy, who was diagnosed at age 26 with Hansen's disease and admitted to the sanatorium when she was 27. At age 71, she was diagnosed as having hepatocellular carcinoma during treatment for hepatitis C, and in 1995, she died of rupture of the cancer-affected liver.

Case 3. An 89-year-old male with lepromatous leprosy, who was diagnosed with Hansen's disease when he was 25, and was admitted to the sanatorium 5 yrs later. At age 81, he was hospitalized because of anorexia, and 3 yrs later in 1995, he died of exacerbation of pneumonia.

Case 4. A 64-year-old male with lepromatous leprosy, whose diagnosis of Hansen's disease was established when he was 14. The next year he was admitted to the sanatorium. At age 62, he was diagnosed with prostate cancer and began hormone therapy. When he was 64 years old, the cancer had metastasized to the stomach, and he died of deterioration of his general condition in 1995.

Case 5. A 79-year-old male with lepromatous leprosy, who was diagnosed as having Hansen's disease when he was 24, was admitted to the sanatorium at age 27. He was diagnosed with a urinary bladder tumor when he was 77 years old and underwent several sessions of transurethral tumor resection. Two years later, when he was 79, he underwent a total cystectomy, but post-operatively developed metastasis of the cancer to the lumbar vertebrae, liver, subcutaneous tissue, and other areas. He died of cancer in the same year in 1996.

Case 6. An 81-year-old male with tuberculoid leprosy was diagnosed at age 21 as having Hansen's disease and admitted to the sanatorium at age 26. When he was 81 years old, he developed chest pain and hy-

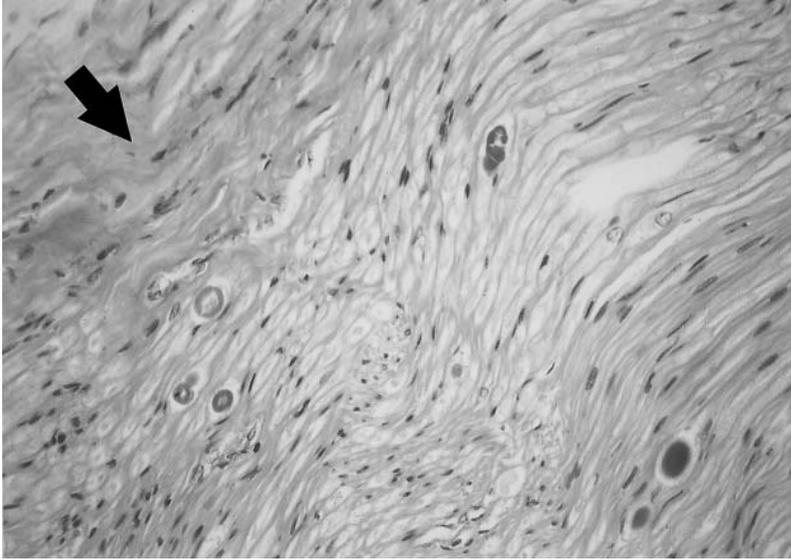


FIG. 1. *Case 1.* Several axonal spherical bodies are visible, some of them granular. These bodies were often detected immediately below the thickened perineurium (arrow). ($\times 400$, PAS).

drothorax associated with thoracic aortic aneurysm, and his condition was complicated by DIC, leading to his death in 1996.

Preparation of specimens. All specimens of the peripheral nerves were fixed in 10% formalin. The specimens collected in 1995 and 1996 were 10 cm long were cut into sections of about 2 cm. They were subjected to HE, PAS, Bodian, Luxol fast blue, amyloid and acid-fast bacterium staining. Formalin-fixed specimens that were found to contain spherical bodies were subjected to PCR assay to determine the relationship of the bodies to *Mycobacterium leprae*. The specimens were also subjected to merge observation (allowing simultaneous observation under both PM and DIC in a common visual field) and electron microscopy.

Merge technique. Non-stained, deparaffinized specimens, 5–7 μm thick, were mounted on acryl-based material. The specimen can be observed simultaneously in a bright visual field under a biomicroscope (BX51, Olympus), a DIC (Olympus) and a PM (Olympus), without the need to move the stage of any of the microscopes. Microscopes capable of magnification up to $\times 1000$ were used. Because DIC and PM (two systems with different properties) share the same polarizing filter (polarizer or analyzer), switching from the DIC to the PM image and vice versa can be done

rapidly, without any distortion in the visual field. At room temperature and under ordinary fluorescent light, polarized images were obtained in the FL and differential-interference images in the BF mode with a digital camera (DP-50, Olympus). The images thus taken were subjected to image analysis using the Photoshop (Adobe) software package. During image analysis, polarizing images were overlapped with differential-interference images of the same

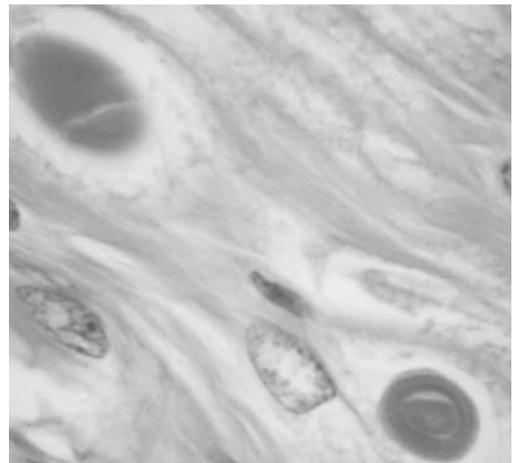


FIG. 2. *Case 1.* Two axonal spherical bodies are visible, one lamellar and the other amorphous. The surrounding myelin sheaths are degenerative. ($\times 1000$, PAS).

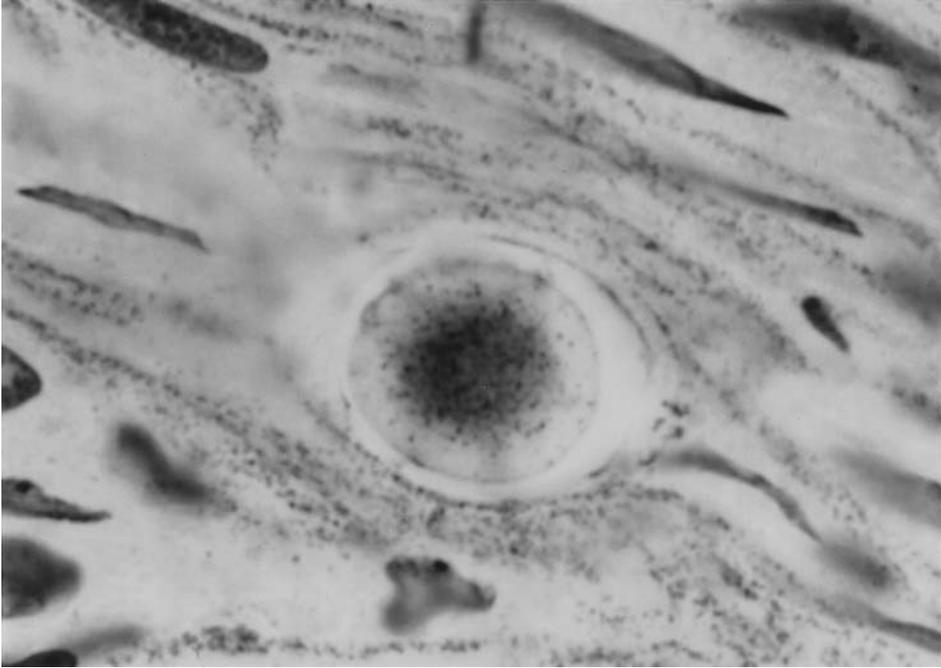


FIG. 3. Case 1. A spherical body is visible in the axon. The silver particles that may be something like a core are in the center. ($\times 1000$, Bodian).

visual field and with the same number of pixels.

Polarizing images can be used to check for polarizing materials, while differential-interference images provide a view of the entire photographed area. Merged images then make it possible to determine the exact location of the polarizing material.

RESULTS

Spherical bodies found within peripheral nerve axons under a light microscope. The diameter of most of the axonal spherical bodies was about $10\ \mu\text{m}$, with one section usually containing 1 or 2 spherical bodies. It was rare for 3 or more bodies to be observed in one section. The bodies were visible in HE-stained sections, but more so in PAS-stained sections (Fig. 1). They were not stained by acid-fast staining and often had a lamellar structure, although some did not show any specific structure. The bodies were often spherical or oval, and some of them were composed of several small granules of irregular size.

A narrow area characterized by irregular swelling was often seen around the spherical bodies within the axons (Fig. 2). The lesions were more frequently seen immediately below the perineurium, and they were

detected in all peripheral nerves examined (median, radial, ulnar, femoral, perinea and sciatic nerves). In Bodian-stained sections, spherical bodies are in the axons and the silver particles are in the center (like a core) (Fig. 3). In Luxol fast blue-stained sections, the spherical bodies did not stain at all (Fig. 4). Their incidence did not correlate with the type of leprosy. These spherical bodies showed no chromatic response to amyloid staining, and, when assayed by PCR, were found to have no relationship to *Mycobacterium leprae* (data not shown).

Findings from transmission electron microscopy. The spherical bodies which appeared to have no specific structure under the light microscope were also amorphous when observed under the electron microscope. The bodies with a lamellar structure under the light microscope, were found under the electron microscope to contain fine powder-like materials with a high electron density in their center (like a core), and show a lamellar structure composed of rings with different electron densities (Fig. 5). The myelin sheaths surrounding the spherical bodies were of irregular thickness (Fig. 6).

Observation with the merge technique. A few of the spherical bodies showed partial polarization, while small areas within

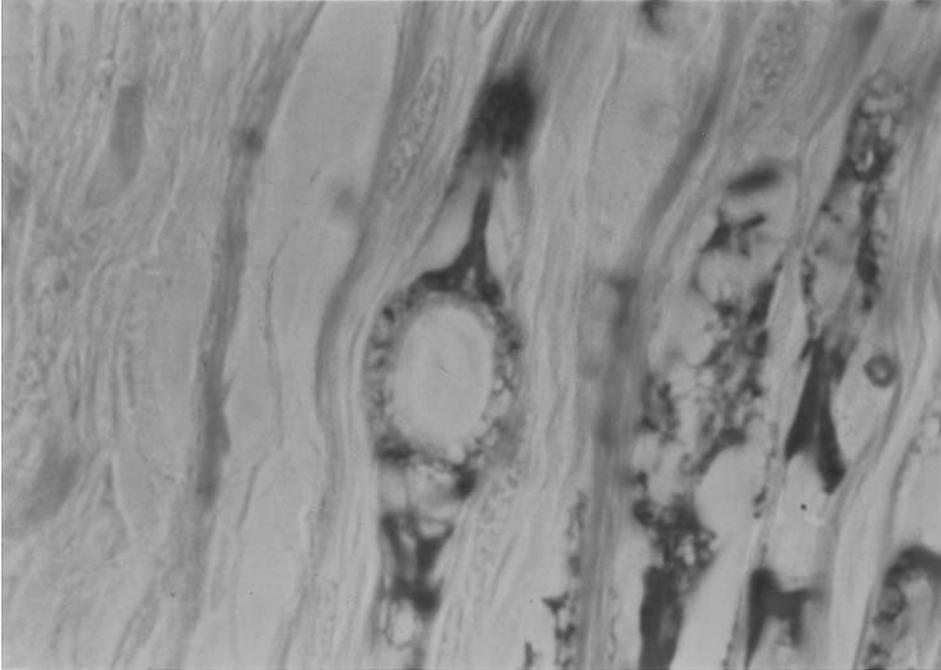


FIG. 4. *Case 1.* A spherical body does not stain at all. ($\times 1000$, Luxol fast-blue).

the myelin sheaths surrounding them were sometimes polarized (Figs. 7, 8). Most spherical bodies had clearly lamellar structure. A number of minute granular or rod-shaped polarized inclusions were visible in the histopathology specimens. The polarization disappeared after treatment of the sections with an alkaline solution.

DISCUSSION

Although a number of reports⁽³⁾ have been published concerning peripheral nerve lesions associated with Hansen's disease, none of them have dealt with axonal spherical bodies. A search of previous findings resembling the spherical bodies we detected in peripheral nerve axons, revealed that the

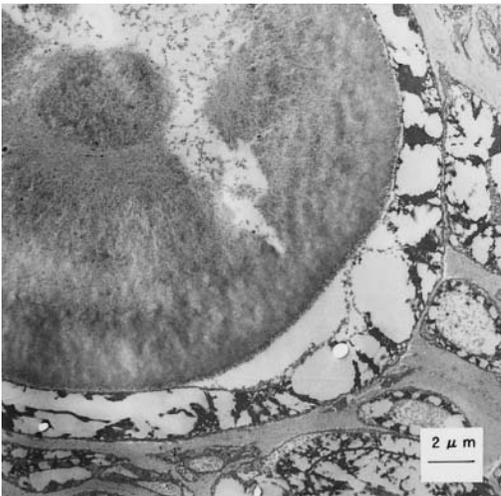


FIG. 5. *Case 1.* Core-like material is visible in the center of the axonal spherical body showing a lamellar structure. (electron micrograph).

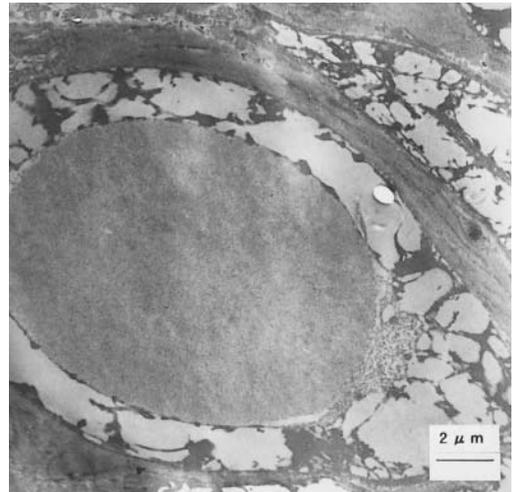


FIG. 6. *Case 1.* An amorphous spherical body is visible within the axon. The surrounding myelin sheaths vary in thickness. (electron micrograph).

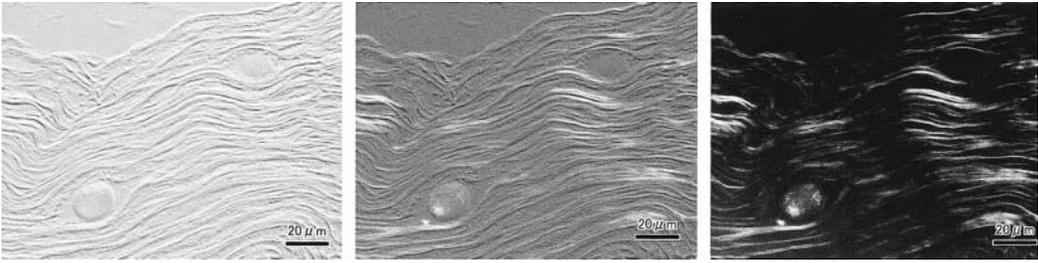


FIG. 7. Case 2. (left: DIC, middle: merge technique, right: PM) Two partially polarized axonal spherical bodies are visible, with the degenerative surrounding myelin sheaths showing some polarized spots. Degenerative connective tissue also shows wave-formed polarization. Polarization of distribution is more evident on the image taken with the merge technique.

ballooning and fragmentation of axons (Bodian stain) shown in Fig. 3 of the report "Pathology of Peripheral Nerve Lesion in Lepromatous Leprosy," published in 1971 by Job⁽⁴⁾, resembled our spherical bodies. The spherical body and "spheroid" which appears pathologically at the time of amyotrophic lateral sclerosis differ from each other in the size and the location.

While tissue specimens, sealed in fixation bottles, were available from not only the six cases included in this study but also from some earlier cases, they had been collected in various ways because post-mortem examinations had been performed by several different pathologists. These earlier specimens were therefore not used for our study because it would be difficult to perform light microscopic observation of all nerve specimens under identical conditions. We do not think that the spherical bodies result from the post mortem change, because the spherical bodies can be seen in both new and old specimens. The patients' illnesses had progressed over more than 10 yrs. We did special stains (Bodian and Luxol fast-blue stain) for the relation between these spherical bodies and the axon or myelin substance. But this special stains showed no

relation between the spherical bodies and the axon or myelin substance.

Under the light microscope, a narrow area characterized by irregular swelling was often observed around the spherical body within the axon. This area seemed to represent a precursor lesion. Among the other areas of the axon, without swelling, the spherical body was often seen in the area where the peripheral perineurium had thickened and become abnormally hard. In this connection, it is interesting that Kimura, *et al.*⁽²⁾ reported that the perineurium of the peripheral nerves appears to be a target of *Mycobacterium leprae*.

When observed under the transmission electron microscope, the spherical lesions often showed a lamellar structure, although some lesions had no specific structure. The lesions contained nothing resembling organelles, nor showed any chromatic response to amyloid staining, indicating that the lesion did not represent amyloid degeneration. The fine powder-like material with a high electron density observed in the central area and resembling a core, differed in hardness from the other areas. This material made it difficult to slice the specimens into sections suitable for electron microscopy.

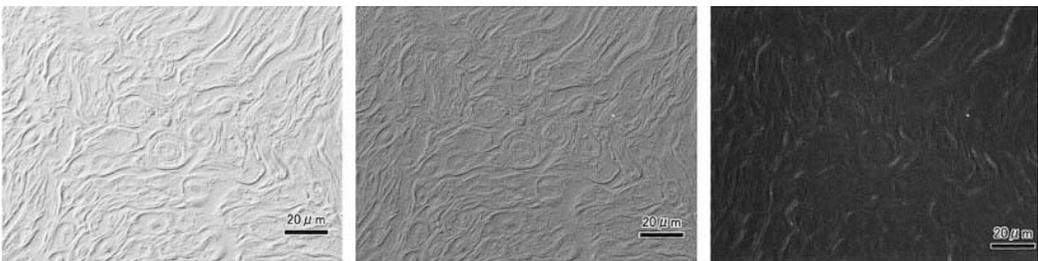


FIG. 8. Case 2. (left: DIC, middle: merge technique, right: PM) A group of axonal spherical bodies with polarization mostly absent, except for some polarized spots.

When observed by the merge technique, the area around the spherical bodies and the myelin sheaths surrounding them was partially polarized in some specimens, although infrequently. Most of the spherical bodies had a clearly lamellar structure, which is consistent with the findings from HE and PAS staining and electron microscopy.

The axonal spherical bodies were clearly PAS-positive, and calcification-like depositions were occasionally seen in their center. These findings, combined with that of the lamellar structure, make it appear likely that the bodies were gradually formed over long periods of time. These bodies were seen in cases of both lepromatous and tuberculoid leprosy. In conclusion, the axonal spherical bodies observed in the study presented here seem to represent lesions gradually formed due to glycoprotein denaturation over long periods of time and are associated with leprosy-caused thickening of the perineurium of peripheral nerves.

Acknowledgment. The authors are indebted to Mr. K. Fukuike (technologist) for his help in preparing the

pathology specimens and to Mr. T. Yamada (Olympus Plaza Osaka) for his help with using the merge technique for taking the DIC and PM pictures. The authors also wish to thank Prof. C. Ide (Department of Anatomy, Kyoto University) for the electron micrographs he prepared for this study and to Dr. K. Saeki (National Sanatorium Oshima Seishoen) for the PCR assay of formalin-fixed peripheral nerve specimens he conducted for this study.

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