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Electron Microscopic Study of Leprosy in a Mangabey Monkey (Natural Infection)[1]

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In 1980 and 1982, Fukunishi, *et al.* ([1, 2]) reported that freeze-etched preparations of tissues from human lepromas, nude mice, and armadillo tissues infected with *Mycobacterium leprae* all demonstrated peculiar spherical droplets around the bacilli in lepra cells. In 1977, Nishiura, *et al.* ([5]) and Takeo, *et al.* (unpublished data) examined the peribacillary substance of various mycobacteria by the freeze-etching technique. Of the 14 species of cultivable mycobacteria examined, none showed the spherical droplets observed around *M. leprae.* The small spherical droplets in lepra cells contain specific glycolipids of *M. leprae* ([3]) and are believed to be unique for *M. leprae* infection, especially the lepromatous leprosy type.

The primary purpose of the present study was to determine, by the freeze-etching technique, the ultrastructural features of lepra cells of a mangabey monkey (*Cercocebus atys*) with naturally acquired leprosy. This mangabey monkey was imported from West Africa in 1975, and housed at Gulf South Research Institute in Louisiana. In 1979, a diagnosis of multibacillary leprosy was made on the basis of clinical and histopathologic findings. After transfer of the monkey to the Delta Regional Primate Research Center in Covington, Louisiana, U.S.A., detailed clinical, histopathologic, immunologic, and bacteriologic studies established that the disease in this animal was caused by *M. leprae.* The animal had never been experimentally inoculated with *M. leprae* ([4]).

MATERIALS AND METHODS

Materials examined in this study were lepromas of a naturally infected mangabey monkey (#A015), a passage mangabey monkey (#A022), a rhesus monkey (#A125), and an African green monkey (#8128) and also leproma and liver of a nine-banded armadillo (#47), as shown in Table 1.

These animals were inoculated with *M. leprae* isolated from a leproma of the naturally infected mangabey monkey by intravenous and intradermal injections. The duration of the leprosy infection in each of these samples was 3.5 years, 3 years, 2.5 years, 2.5 years, and 13 months, respectively, as shown in Table 2. These samples were observed by electron microscopy, especially by the freeze-etching technique.

Portions of the lepromas and livers were fixed with 3% glutaraldehyde in 0.06 M phosphate buffer, pH 7.4, for 24–48 hr at 4°C for the study of ultrathin sections. The tissues were post-fixed with 2% OsO_4 in distilled water after 24 hr at 4°C. After dehydration the tissues were embedded in Spurr low-viscosity medium, cut by an ultramicrotome with glass knives, and stained with uranyl acetate and lead nitrate.

The tissues for study by the freeze-etching technique were fixed with 3% glutaraldehyde under the same conditions as those for ultrathin sectioning, and then immersed in 20% glycerol for 24–48 hr at 4°C. Other procedures were the same as described previously by Nishiura, *et al.* ([5]).

RESULTS

The size, shape, and ultrastructural features of leprosy bacilli in phagolysosomes of macrophages in all of these samples (two mangabey monkeys, a rhesus monkey, a

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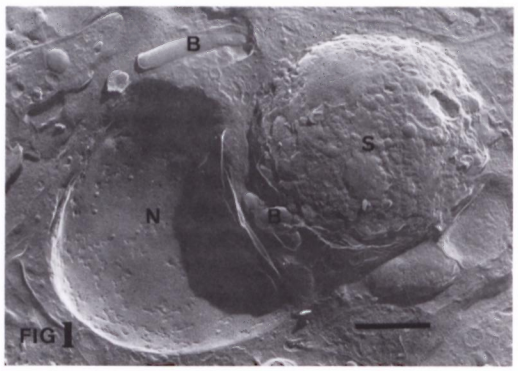

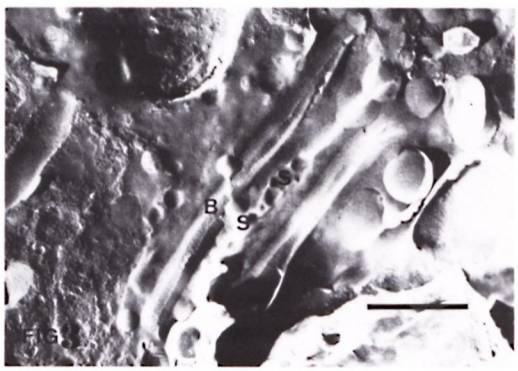

FIG. 1. Large amounts of small spherical droplets and leprosy bacilli observed in the lepra cells of the naturally infected mangabey monkey (#A015) (×20,000).

B = leprosy bacilli; S = small spherical droplets; N = nucleus; ➡ = lysosomal membrane; ➞ = band structures; scale = 1 μm.

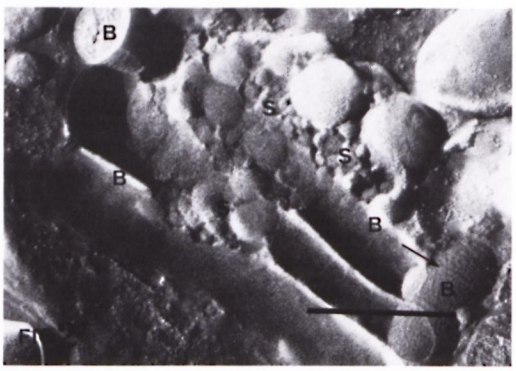

FIG. 2. Small spherical droplets around leprosy bacilli with band structures on the smooth cell wall surfaces observed in the lepra cells of a passage mangabey monkey (#A022). These leprosy bacilli make a globus (×40,000). (See Fig. 1 for symbol legend.)

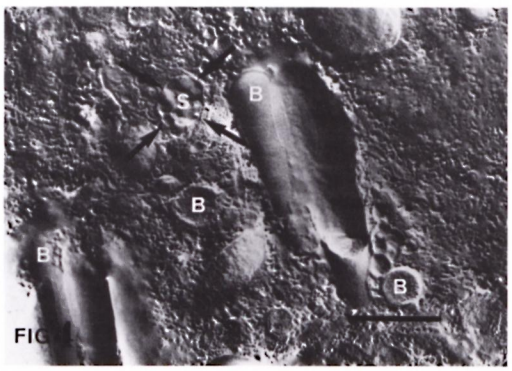

FIG. 3. Small spherical droplets around the leprosy bacilli observed in the lepra cells of a passage mangabey monkey (#A022). These leprosy bacilli are long and slender (×27,000). (See Fig. 1 for symbol legend.)

FIG. 4. Leprosy bacilli with band structures on the smooth cell wall surfaces and small spherical droplets inside a phagolysosome observed in the lepra cells of a rhesus monkey (#A125) (×25,000). (See Fig. 1 for symbol legend.)

TABLE 1. *Tissues studied by electron microscopy.*

Animal no.	Species of animal	Specimen
A015	Sooty mangabey monkey (*Cercocebus atys*)	Left lateral calf (biopsy)
A022	Sooty mangabey monkey (*Cercocebus atys*)	Right lateral calf (biopsy)
A125	Rhesus monkey (*Macaca mulatta*)	Left forearm (biopsy)
8178	African green monkey (*Cercopithecus aethiops*)	Ear (biopsy)
47	Nine-banded armadillo (*Dasypus novemcinctus*)	Subcutaneous leproma and liver (autopsy)

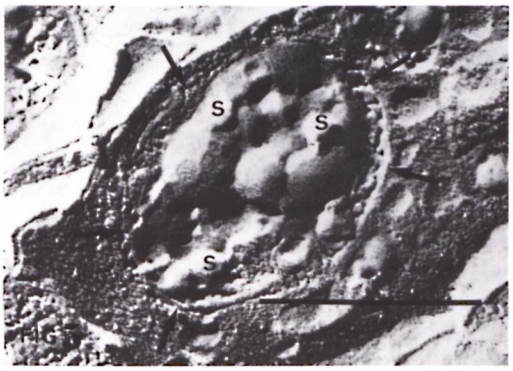

FIG. 5. Small spherical droplets inside a phagolysosome observed in the lepra cells of a rhesus monkey (#A125). The finding is typical of an intracytoplasmic foamy structure (×60,000). (See Fig. 1 for symbol legend.)

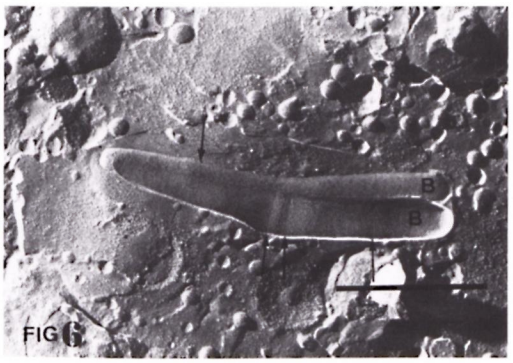

FIG. 6. Two leprosy bacilli observed in a lepra cell of a nine-banded armadillo (#47). These leprosy bacilli are long and slender and have band structures on the smooth cell wall surfaces. A dividing leprosy bacillus is observed here, and band structures are found on both sides of the division site (×42,000). (See Fig. 1 for symbol legend.)

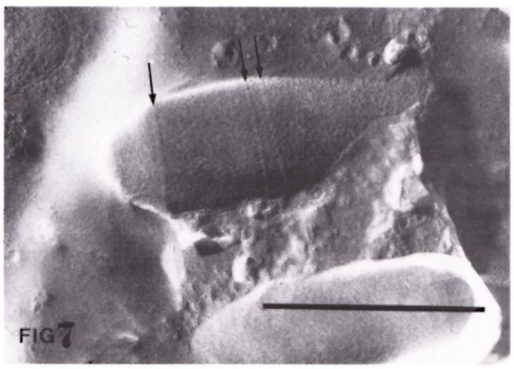

FIG. 7. A leprosy bacillus with band structures on the smooth cell wall surface observed in a lepra cell of a nine-banded armadillo (#47). These band structures are formed of double strings (×60,000). (See Fig. 1 for symbol legend.)

green monkey, and a nine-banded armadillo) were essentially the same as those in human lepromas and in nude mice and armadillo tissues inoculated with *M. leprae* isolated from human lepromas.

In ultrathin sections, there were typical electron-transparent zones around the multiplying leprosy bacilli in the lepra cells of all the samples except those from the African green monkey.

In freeze-etched specimens, distinct accumulations of small spherical droplets were observed around the leprosy bacilli inside the lepra cells of all samples (Figs. 1–5), but they were scarce in the specimen from the green monkey. The bacilli in all the samples were long and slender (Figs. 1–3, 6) and had band structures on the smooth cell wall surfaces (Figs. 2, 6, and 7). The bacilli were indistinguishable from *M. leprae*. Results are summarized in Table 3.

DISCUSSION

A large number of small spherical droplets were observed around the leprosy bacilli in the lepra cells of all the samples studied, but in the green monkey, the spherical droplets were scarce. The morphologic features of the bacilli in all the specimens from the four animal species were similar to those of *M. leprae*.

The observations on these specimens provide additional evidence to support the belief that the etiologic agent of leprosy in the naturally infected mangabey monkey is *M. leprae*. The findings further demonstrate that the type of leprosy in the first mangabey monkey (#A015), the passage mangabey monkey (#A022), the rhesus monkey (#A125), and the armadillo (#47) are like that of human leprosy and those of experimental leprosy in nude mice and armadillos. The leprosy in the green monkey is in an early stage of development and cannot be precisely classified.

SUMMARY

Ultrastructural features of the leproma of a) a naturally infected mangabey monkey,

TABLE 2. *Inoculation and clinical information on animals studied.*

Animal no. and species	Source of inoculum	No. of AFB inoculated	Duration of infection
A015 Sooty mangabey monkey (naturally acquired leprosy infection)	—	—	3.5 years[a]
A022 Passage sooty mangabey monkey	A015	1.2×10^9 iv 1.2×10^9 id	36 months
A125 Rhesus monkey	A015	1.0×10^8 iv 2.0×10^8 id (ears and face)	27 months
8178 African green monkey	A015	1.0×10^8 iv 2.0×10^8 id (ears and face)	27 months
47 Nine-banded armadillo	A015	1.3×10^9 iv	13 months

[a] Unknown with certainty; diagnosed in 1979.

TABLE 3. *Ultrastructural features of lesions (freeze etching) in animals studied.*

Animal no. and species	Shape of bacilli	Band structure	Globi	Spherical droplets
A015 Mangabey monkey (natural infection)	Long and slender	Positive	Positive	Positive
A022 Passage mangabey monkey	Long and slender	Positive	Positive	Positive
A125 Rhesus monkey	Long and slender	Positive	Positive	Positive
8178 African green monkey	Long and slender	Positive	Scarce	Scarce
47 Nine-banded armadillo	Long and slender	Positive	Positive	Positive

and lepromas and liver of b) a passage mangabey monkey, c) a rhesus monkey, d) an African green monkey, and e) a nine-banded armadillo inoculated with leprosy bacilli isolated from the leproma of a naturally infected mangabey monkey were studied by the freeze-etching technique. The size, shape, and ultrastructural features of leprosy bacilli in the phagolysosomes of macrophages in all of these samples were essentially the same as those in humans, nude mice, and armadillos inoculated with human *Mycobacterium leprae.* Distinct accumulations of small spherical droplets were observed around leprosy bacilli inside lepra cells of all the samples but were scarce in the specimen from the green monkey. The bacilli in all samples were long and slender, and had band structures on the smooth cell wall surfaces. The bacilli were indistinguishable from *M. leprae.*

RESUMEN

Usando la técnica de "impresión por congelación" se hizo un estudio ultraestructural de los lepromas obtenidos de monos "mangabey" infectados en forma natural y de los lepromas e hígados obtenidos (a) de monos "mangabey" inoculados con material derivado de los monos con la infección natural, (b) de mono rhesus, (c) de un mono verde africano, y (d) de un armadillo de nueve bandas inoculado con bacilos aislados del mono "mangabey" infectado en forma natural. El tamaño, la forma y la ultraestructura de los bacilos de la lepra encontrados en los fagolisosomas de los macrófagos de todas las muestras fueron esencialmente iguales a los encontrados en humanos, en ratones desnudos y en armadillos inoculados con *My-*

cobacterium leprae de orígen humano. Alrrededor de los bacilos contenidos en los histiocitos de todos los especímenes estudiados se observaron acumulaciones de pequeñas gotas o vesículas esféricas muy características las cuales fueron escasas en las especímenes obtenidos del mono verde. En todos los casos, los bacilos fueron largos y delgados y tuvieron estructuras en forma de banda sobre la superficie lisa de la pared celular. Los bacilos fueron indistinguibles del *M. leprae.*

RÉSUMÉ

Les caractéristiques de l'ultrastructure du léprome ont été étudiées par une méthode de cryodécapage dans les lépromes d'un singe mangabey infecté naturellement, de même que dans les lépromes et le foie d'un singe mangabey inoculé par passage, d'un singe rhésus, d'un singe vert africain, et de tatous à neuf bandes inoculés avec des bacilles de la lèpre isolés d'un léprome provenant d'un singe mangabey infecté naturellement. On a constaté que la dimension, la forme, et les caractéristiques de l'ultrastructure des bacilles de la lèpre dans les phagolysosomes de macrophages dans tous ces échantillons ont été essentiellement semblables à ceux observés chez les hommes, les souris glabres, et des tatous inoculés avec *Mycobacterium leprae* humain. Des accumulations nettes de petites gouttelettes sphériques ont été observées autour des bacilles de la lèpre, à l'intérieur des cellules lépreuses, dans tous les échantillons; elles étaient cependant rares dans l'échantillon provenant du singe vert. Dans tous les échantillons, les bacilles étaient longs et minces; ils présentaient des structures en bandes sur la surface des parois des cellules lisses. Il n'était pas possible de faire une distinction avec *M. leprae.*

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REFERENCES

1. Fukunishi, Y., Meyers, W. M., Walsh, G. P., Johnson, F. B., Binford, C. H., Okada, S. and Nishiura, M. Ultrastructural features of the multiplication of leprosy bacilli in macrophages of armadillos inoculated with *M. leprae.* Abstract in Int. J. Lepr. **48** (1980) 494.
2. Fukunishi, Y., Okada, S., Nishiura, M. and Kohsaka, K. Ultrastructural features of the multiplication of human and murine leprosy bacilli in macrophages of nude mice. Int. J. Lepr. **50** (1982) 68–75.
3. Fukunishi, Y., Okada, S., Nishiura, M. and Kosaka, T. Electronmicroscopic and biochemical studies of the peribacillary substance of *M. leprae.* Abstract in Int. J. Lepr. **49** (1981) 499.
4. Meyers, W. M., Walsh, G. P., Brown, H. L., Fukunishi, Y., Binford, C. H., Gerone, P. J. and Wolf, R. H. Naturally-acquired leprosy in a mangabey monkey (*Cercocebus* sp.). Abstract in Int. J. Lepr. **48** (1980) 495–496.
5. Nishiura, M., Izumi, S., Mori, T. and Nonaka, T. Freeze-etching study of human and murine leprosy bacilli. Int. J. Lepr. **45** (1977) 248–254.
6. Yamamoto, T., Nishiura, M., Harada, N. and Imaeda, T. Electron microscopy of ultra-thin sections of lepra cells and *Mycobacterium leprae.* Int. J. Lepr. **26** (1958) 1–8.