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Electron Microscopic Findings of Transverse Fission of *M. leprae* by Freeze-etching Methods[1]

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Reproduction by transverse fission or budding are thought of as the methods of multiplication of *Mycobacterium leprae in vivo*. The detailed process of multiplication of *M. leprae* has not been elucidated however because standard culture of *M. leprae in vitro* has so far not been achieved.

In 1983 Mor ([7]), reporting on the electron microscopic findings of *M. leprae* in the macrophages of normal and immune-deficient mice, found that *M. leprae* multiply freely in the cytoplasm of the macrophages of infected mice, but *M. leprae* cells resident within the phagosomes of the macrophages are dead.

In 1981 ([4]), 1982 ([5]), and 1984 ([2,3]), Fukunishi, *et al.* reported freeze-etching studies of human, nude mouse, armadillo, and monkey lepra cells, and considered that *M. leprae* multiplied inside the phagolysosomes, producing small spherical droplets around themselves.

In this paper, the freeze-etching findings of multiplication by transverse fission of *M. leprae* inside the phagolysosomes of lepra cells of some animal models are reported.

MATERIALS AND METHODS

Samples used for the freeze-etching technique were: lepromas of nude mice inoculated with *M. leprae* isolated from human lepromas (Kyoto University, Japan [4,5]); lepromas and livers of nine-banded armadillos inoculated with *M. leprae* isolated from a human leproma (Armed Forces Institute of Pathology [AFIP], U.S.A. [3]); a leproma and liver of a nine-banded armadillo with naturally acquired leprosy-like disease (AFIP[1]) and leproma and liver of a nine-banded armadillo inoculated with *M. leprae* isolated from the mangabey monkey with naturally acquired leprosy infection (AFIP [2,6]).

Electron microscopy procedures were: the lepromas and livers were fixed with 3% glutaraldehyde in 0.06 M phosphate buffer (pH 7.4) for 24–48 hr, and then immersed in 20–40% glycerol for 1–2 days at 4°C. Other procedures were the same as described previously ([8]).

RESULTS

In a well-developed leproma, elongation of the leprosy bacilli is observed frequently as shown in Figure 1. The leprosy bacillus shown in Figure 1 is more slender and longer than usual.

Figure 2 shows a leprosy bacillus which has started to divide by transverse fission. The fission line is observed at the center of the smooth cell wall surface of the bacillus, and band structures are observed on both sides of the line.

Figures 3 and 4 show leprosy bacilli just before the end of transverse fission inside the phagolysosomes of lepra cells. Band structures are again observed on both sides of the fission line. Small spherical droplets are seen around the bacilli.

Figure 5 shows two leprosy bacilli which have completed division by transverse fission inside the phagolysosome. Small spherical droplets are observed around them.

Band structures are usually seen on both sides of the fission line at the time of multiplication by transverse fission. It is not clear whether these band structures appear before or after the appearance of the fission line.

Multiplication of leprosy bacilli by transverse fission is observed generally inside the phagolysosomes of lepra cells. The phagolysosomal membrane is seen clearly if the samples are well preserved.

The procedures of the transverse fission, illustrated by Figure 6, show the same pattern in all lepra cells studied: a) nude mice and nine-banded armadillos inoculated with *M. leprae* isolated from human leproma, b) nine-banded armadillos with naturally ac-

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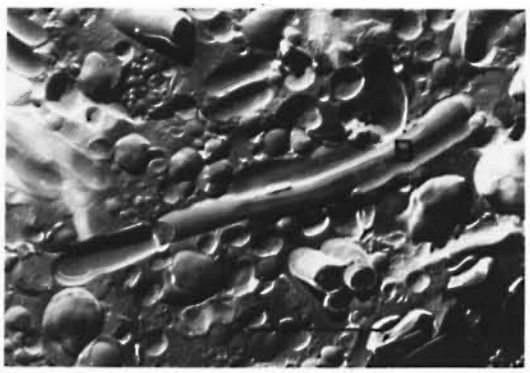

FIG. 1. Freeze-etching of a lepra cell of an armadillo inoculated with *M. leprae* isolated from a human leproma. Note slender elongation of the leprosy bacillus. B = leprosy bacillus (original magnification ×35,000).

FIG. 2. Freeze-etching of a lepra cell of an armadillo inoculated with *M. leprae* isolated from a mangabey monkey with naturally acquired leprosy infection. A leprosy bacillus which has started to divide by transverse fission is observed. Note fission line at center of the bacillus and band structures on both sides of the line. B = leprosy bacillus, thin arrow = band structure, semi-thick arrow = fission line, thick arrow = lysosomal membrane (original magnification ×31,000).

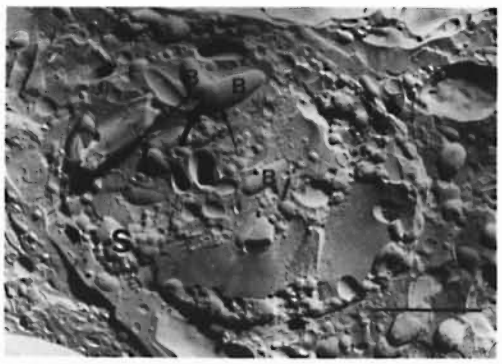

FIG. 3. Freeze-etching of a lepra cell of an armadillo with naturally acquired leprosy-like disease. The leprosy bacillus is shown just before the end of transverse fission inside the phagolysosome of a lepra cell. Band structures are seen on both sides of the fission line. Small spherical droplets are around the leprosy bacilli. B = leprosy bacillus, S = spherical droplets, thin arrow = band structure, semi-thick arrow = fission line, thick arrow = lysosomal membrane (original magnification ×29,000).

quired leprosy-like disease, and c) the nine-banded armadillo inoculated with *M. leprae* isolated from the mangabey monkey with naturally acquired leprosy infection.

DISCUSSION

Up to now, it was unclear whether or not leprosy bacilli multiply inside the phagolysosome of the lepra cell. Generally speaking, leprosy bacilli are observed by electron microscopy inside phagolysosomes of the macrophage, Schwann cell, striated muscle cell, and endothelial cell of small blood vessels. On the other hand, many leprosy bacilli are also seen in the intercellular space outside these cells, and when these leprosy bacilli are observed by the freeze-etching technique, lysosomal membrane-like segments are frequently found clinging to them. From these freeze-etching findings, it seems most likely that these extracellular leprosy bacilli originally came from the phagolysosomes of macrophages whose lifespan came to an end.

In this paper, images of multiplication by transverse fission of *M. leprae* in a) the lepra cells of nude mice and nine-banded armadillos inoculated with *M. leprae* isolated from human lepromas, b) nine-banded armadillos with naturally acquired leprosy-like disease, and c) a nine-banded armadillo inoculated with *M. leprae* isolated from the mangabey monkey with naturally acquired infection, are shown. These were almost always observed inside phagolysosomes. The armadillo liver used for the freeze-etching observation shown in Figure 2, in which the lysosomal membrane was not clear, was frozen once and then fixed with 3% glutaraldehyde after thawing for about 1 hour. Accordingly, the preservation of the sample for the freeze-etching technique was not good.

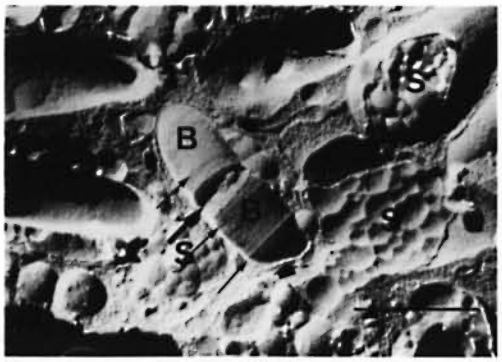

FIG. 4. Freeze-etching of a lepra cell of a nude mouse inoculated with *M. leprae* isolated from a human leproma. The bacillus is shown just before the end of transverse fission inside the phagolysosome of a lepra cell. The fission line is at center of the smooth cell wall surface of the bacillus. Band structures are seen on both sides of the line (original magnification ×33,000). (See Fig. 3 for abbreviations.)

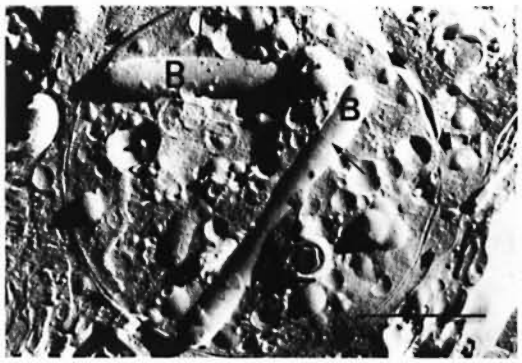

FIG. 5. Freeze-etching of a lepra cell of a nude mouse inoculated with *M. leprae* isolated from a human leproma. Two leprosy bacilli which have completed division by transverse fission are shown inside the phagolysosome (original magnification ×33,000). (See Fig. 3 for abbreviations.)

These results indicate that *M. leprae* multiply by transverse fission *in vivo* inside phagolysosomes of macrophages and other cells of nude mice and nine-banded armadillos.

SUMMARY

The structures of multiplication by transverse fission found in the lepromas and livers of nude mice and nine-banded armadillos inoculated with *Mycobacterium leprae* isolated from human lepromas, a nine-banded armadillo with naturally acquired leprosy-like disease, and a nine-banded armadillo inoculated with *M. leprae* isolated from the mangabey monkey with naturally acquired leprosy infection are described.

The images of multiplication by transverse fission of *M. leprae* were almost always found inside the phagolysosomes of lepra cells. At the time of multiplication by transverse fission, band structures were observed generally at both sides of the fission line. According to these results, multiplication of *M. leprae* by transverse fission is done inside the phagolysosomes of lepra cells of nude mice and nine-banded armadillos.

RESUMEN

Se describen las estructuras relacioandas con multiplicación por fisión transversa, encontradas en los

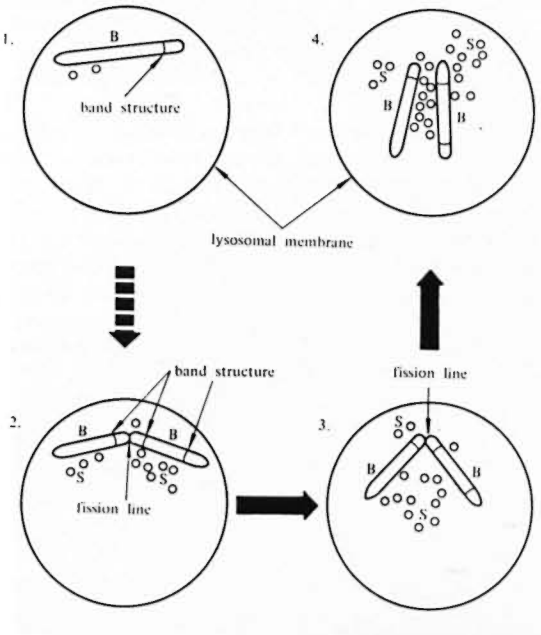

FIG. 6. Procedures of transverse fission. Division by transverse fission of leprosy bacilli is observed generally inside the phagolysosomes of lepra cells. The phagolysosomal membrane is seen clearly if samples are well preserved. Band structures are usually observed on both sides of the fission line at the time of multiplication by transverse fission. It is not clear whether band structures appear before or after the appearance of the fission line on the smooth cell wall surface of the bacilli. Small spherical droplets are accumulated around the multiplying leprosy bacilli. B = leprosy bacillus, S = spherical droplets.

lepromas e hígados de ratónes desnudos y de armadillos de 9 bandas inoculados con *Mycobacterium leprae* aislados de lepromas humanos, en un armadillo con "lepra" natural, y en un armadillo inoculado con *M. leprae* aislado de un mono mangabey con "lepra" natural.

Las imágenes de multiplicación por fisión transversa del *M. leprae* casi siempre fueron encontradas dentro de los fagolisosomas de las células de la lepra. En general, siempre se observaron estructuras en banda a ambos lados de la línea de fisión cuando ocurrió multiplicación por fisión transversa. De acuerdo a estos resultados, la multiplicación por fisión transversa del *M. leprae* ocurre dentro de los fagolisosomas de las células de la lepra de los ratones desnudos y de los armadillos de 9 bandas.

RÉSUMÉ

On décrit les structures observées au cours de la multiplication par division transverse dans les lépromes et dans le foie de souris glabres et de tatous à neuf bandes inoculés par des bacilles *Mycobacterium leprae* récoltés à partir respectivement de lépromes humains, d'un tatou à neuf bandes atteint d'une maladie semblable à la lèpre mais acquise naturellement, et d'un tatou à neuf bandes inoculé par des bacilles *M. leprae* isolés d'un singe mangabey ayant acquis naturellement une infection lépreuse.

Les images de multiplication par division transverse de *M. leprae* ont presque toujours été observées à l'intérieur des phagolysosomes des cellules lépreuses. Au moment de la multiplication par division transverse, des structures en rubans ont généralement été observées des deux côtés de la ligne de division. On peut déduire de ces résultats que la multiplication de *M. leprae* par division transverse se produit à l'intérieur des phagolysosomes des cellules de lèpre chez la souris glabre et chez le tatou à neuf bandes.

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