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Electron Microscopic Findings of the Peripheral Nerve Lesions of Nude Mice Inoculated with *M. leprae*[1]

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Successful inoculation of various experimental animals with *Mycobacterium leprae* has revealed that the invading bacilli cause lepromatoid peripheral nerve lesions in the animals in which they multiply. Job, in 1970 ([6]) and 1971 ([7]), published electron microscopic findings of the peripheral nerve lesions in human lepromatous leprosy, and described that *M. leprae* were frequently present in large numbers in Schwann cells, macrophages and endothelial cells, and occasionally present in perineurial cells.

In 1974, Boddingius ([2]) reported on the electron microscopic findings of the sciatic nerves of mice inoculated with *M. leprae,* and found bacilli in axons and Schwann cells of unmyelinated fibers and in the endoneurial macrophages. Also in 1974, Yoshizumi, *et al.* ([11]) published a light and electron microscopic study of peripheral nerve lesions of the nine-banded armadillo inoculated with *M. leprae* and found bacilli in macrophages, endothelial cells, perineurial cells, and Schwann cells of unmyelinated fibers. Peripheral nerve lesions have also been reported in naturally acquired, leprosy-like disease of the armadillo ([1, 9]).

In 1983, Chehl, *et al.* ([3]) reported the growth of *M. leprae* in nude mice, and described massive foot pad enlargement with eventual dissemination of the bacilli in virtually all organs except the central nervous system. In the peripheral nervous system, they found leprosy bacilli in Schwann cells and in perineurial cells.

This paper deals with changes of the peripheral nerves in nude mice inoculated with *M. leprae* and examined by electron microscopy.

MATERIALS AND METHODS

Studies on leprosy in nude mice were initiated at the Leprosy Research Laboratory, Kyoto University, Japan, in 1979.

When the foot pads of nude mice became enlarged as the lesions developed, the mice were sacrificed, and portions of the foot pads were fixed with 3% glutaraldehyde in 0.06 M phosphate buffer (pH 7.4) for 24–48 hr, washed three times with the buffer, and postfixed with 2% OsO_4 in distilled water for 14–24 hr at 4°C. After dehydration with a graded ethanol series, the tissues were embedded in Spurr or methacrylate, cut by an ultramicrotome with glass knives, and then stained with uranyl acetate and lead citrate for ultrathin section study and with methylene blue for semithin section study.

The present study is based on the findings of five lepromas of foot pads (from 1 year to 1 year and 8 months after the inoculations) and three sciatic nerves of the inoculated nude mice.

RESULTS

Leprosy bacilli were found in perineurial cells, endoneurial macrophages, Schwann cells, and axons of myelinated fibers. Bacilli were also found in the endothelial cells of the capillaries of the peripheral nerves.

Distinct foamy changes ([4, 5, 8, 10]) were seen in perineurial cells and in Schwann cells of the myelinated nerve fibers. However, it was interesting to note that there were few degenerating bacilli and that almost all leprosy bacilli were observed as solid.

Figure 1 shows leprosy bacilli in the endothelial cell of a small blood vessel in the peripheral nerve inside the foot pad leproma. Figure 2 shows intracytoplasmic foamy structures ([4, 5, 8, 10]) in the perineurial cells and in macrophages outside the perineurial cells of an inoculated foot pad. A leprosy bacillus is observed in the axoplasm also.

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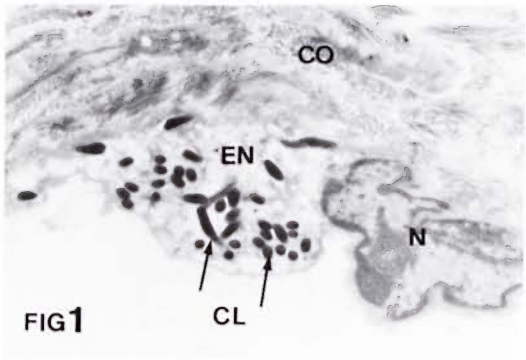

FIG. 1. Leprosy bacilli in an endothelial cell of a capillary in the peripheral nerve inside a foot pad leproma. Almost all the leprosy bacilli appear solid.

AP = axoplasm; CL = capillary lumen; CO = collagen fiber; EN = endothelial cell; ET = electron-transparent zone; M = myelin; N = nucleus; PN = perineurial cell; RBC = red blood cell; SC = Schwann cell; SM = striated cell; Arrow = leprosy bacillus.

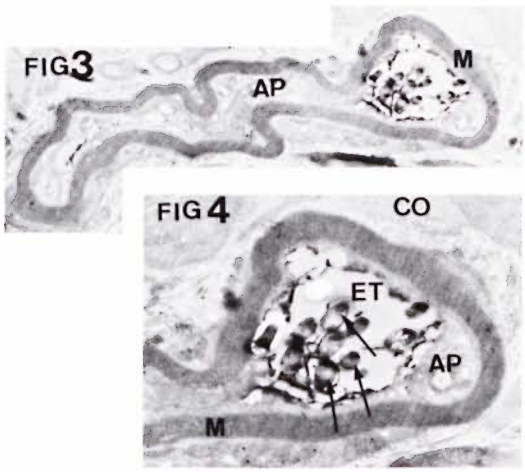

FIG. 3. AND FIG. 4. An intracytoplasmic foamy structure in the axoplasm of a myelinated nerve of an inoculated foot pad. Many leprosy bacilli are observed in the axoplasm, with the axolemma surrounding the axoplasm. (See Fig. 1 for abbreviations.)

Axolemma surrounding the axoplasm can also be seen.

Figures 3 and 4 show an intracytoplasmic foamy structure in the axoplasm of a myelinated nerve of an inoculated foot pad. Many leprosy bacilli are observed in the axoplasm, with axolemma surrounding the axoplasm.

Figure 5 shows many leprosy bacilli in the perineurial space and in the macrophages outside the nerves of an inoculated foot pad.

Figure 6 shows an intracytoplasmic foamy structure in the Schwann cell observed in an inoculated foot pad. The electron-transparent zone is distinct around the leprosy bacilli.

Figure 7 shows leprosy bacilli in an endoneurial macrophage of a sciatic nerve of the limb on the inoculated side.

Perineurial cells contained the largest number of leprosy bacilli in the peripheral nerves. It is interesting that intra-axonal bacilli were found in the myelinated axons in the nude mice.

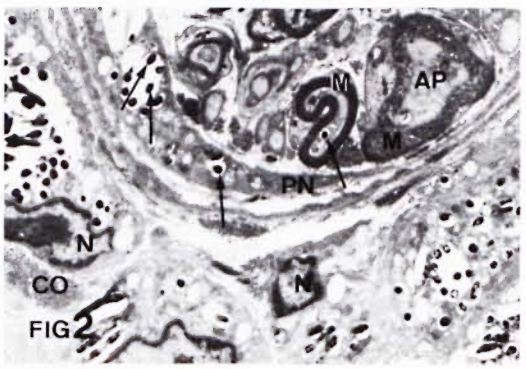

FIG. 2. Intracytoplasmic foamy structures in perineurial cells and macrophages of an inoculated foot pad. A leprosy bacillus is observed in the axoplasm. The axolamma surrounding the axoplasm can also be seen. Almost all the leprosy bacilli appear solid. (See Fig. 1 for abbreviations.)

FIG. 5. Many leprosy bacilli in the perineurial space and in the macrophages outside the nerves of an inoculated foot pad. (See Fig. 1 for abbreviations.)

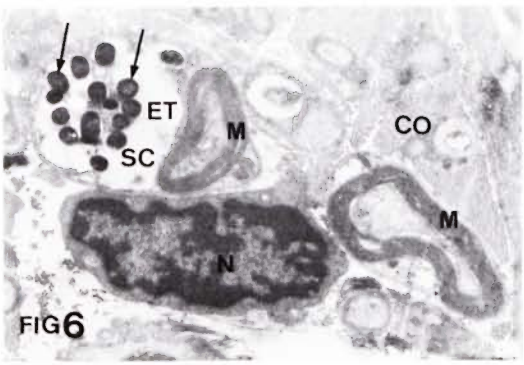

FIG. 6. An intracytoplasmic foamy structure in a Schwann cell observed in an inoculated foot pad. An electron-transparent zone is observed distinctly around the leprosy bacilli. Almost all the bacilli appear solid. (See Fig. 1 for abbreviations.)

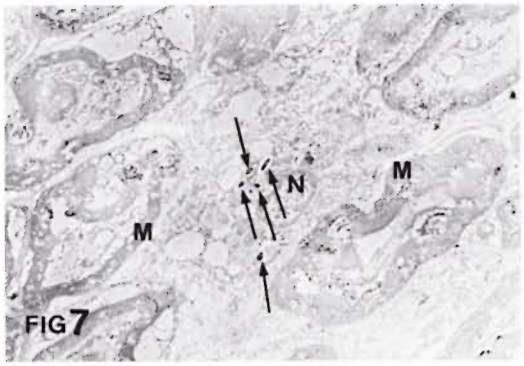

FIG. 7. Leprosy bacilli in an endoneurial macrophage of a sciatic nerve of a limb on the inoculated side. (See Fig. 1 for abbreviations.)

DISCUSSION

The fundamental pathological features of the peripheral nerve lesions in nude mice inoculated with *M. leprae* were almost the same as those ([1-3, 6, 7, 9, 11]) of other mice and armadillos inoculated with *M. leprae* and those of human lepromatous leprosy.

In this study, a large number of leprosy bacilli were observed in the endothelial cells of capillaries, perineurial cells, axoplasms of myelinated nerves, Schwann cells, and endoneurial macrophages of inoculated foot pads, and in the endoneurial macrophages of the sciatic nerves. Intracytoplasmic foamy structures were also observed in these cells, but marked fibrosis, hypertrophy or degeneration of Schwann cells, demyelination, axonal damage, or irreversible destruction of nerve architecture were not observed clearly.

From these observations it would seem that nude mice inoculated with *M. leprae* are suitable models for the study of the early lesions of the peripheral nervous system in lepromatous leprosy.

Since the specimens used were lepromas of nude mice foot pads, it seems probable that in foot pad lesions perineurial cells are the first target of bacilli invading from neighboring lepromas. Figure 2 shows a large number of leprosy bacilli in the perineurial cells and in macrophages outside the perineurial cells, but they are not as numerous in the nerve parenchyma.

In the proximal portion of the sciatic nerves of a limb of the inoculated side, bacilli were found in the macrophages of the endoneurial space, but no bacillus was found in the perineurial cells of the sciatic nerves. This also supports the theory that the route of invasion of bacilli into perineurial cells in the lesion of a foot pad may be permeation from a neighboring lesion.

SUMMARY

Peripheral nerve lesions of nude mice inoculated with *Mycobacterium leprae* were examined from 1 year to 1 year and 8 months post-inoculation. Leprosy bacilli and intracytoplasmic foamy structures were found in the perineurial cells, endothelial cells of capillaries, Schwann cells, and axons of the myelinated nerve fibers inside the lepromas of foot pads. In the proximal portion of the sciatic nerve, bacilli were found chiefly in the macrophages of the endoneurial space. Bacilli were not found in the perineurial cells of the sciatic nerves. Marked fibrosis, hypertrophy or degeneration of Schwann cells, demyelination, axonal damage or irreversible destruction of nerve architecture were not observed clearly.

Therefore, it is thought that nude mice inoculated with *M. leprae* are suitable models for the study of the early peripheral nerve lesions of lepromatous leprosy.

RESUMEN

Se examinaron las lesiones en nervios periféricos que aparecieron en ratones desnudos de 1 a 1.8 años después de la inoculación con *Mycobacterium leprae*. Se encontraron bacilos de la lepra y estructuras espumosas

intracitoplásmicas en las células perineuriales, en las células endoteliales de los capilares, en las células de Schwann, y en los axones de las fibras nerviosas mielinizadas dentro de los lepromas de los cojinetes plantares. En la porción proximal del nervio sciático los bacilos se encontraron principalmente en los macrófagos del espacio endoneurial. No se encontraron bacilos en las células perineuriales de los nervios sciáticos. No se observaron (al menos en forma clara) ninguno de los siguientes cambios: fibrosis marcada, hipertrofia o degeneración de células de Schwann, desmielinización, daño axonal o destrucción irreversible de la arquitectura de los nervios.

Por lo tanto se piensa que los ratones desnudos inoculados con *M. leprae* son modelos adecuados para el estudio de las lesiones tempranas en nervious periféricos de la lepra lepromatosa.

RÉSUMÉ

Les lésions nerveuses périphériques ont été examinées chez des souris glabres inoculées avec *Mycobacterium leprae,* de 1 an à 1 an et 8 mois après l'inoculation. On a observé des bacilles de la lèpre et des structures spumeuses intracytoplasmiques dans les cellules perineurales, les cellules endothéliales des capillaires, les cellules de Schwann, et les axones des fibres nerveuses myélinisées à l'intérieur des lépromes des coussinets plantaires. Dans la partie proximale du nerf sciatique, les bacilles étaient répartis principalement dans les macrophages de l'espace endoneural. Aucune bacille n'a été décelé dans les cellules périneurales des nerfs sciatiques. Il n'a pas été possible de mettre clairement en évidence une fibrose marquée, une hypertrophie ou une dégénerescence des cellules de Schwann, une démyélination, des lésions au niveau des axones, ou une destruction irréversible de la texture nerveuse.

On estime dès lors que les souris glabres inoculées par *M. leprae* constituent des modèles appropriés pour l'étude des lésions nerveuses périphériques précoces dans la lèpre lépromateuse.

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