INTERNATIONAL JOURNAL OF LEPROSY

Volume 56, Number 2
Printed in the U.S.A.

Lepromatous Meningoencephalitis in the Nine-banded Armadillo (*Dasypus novemcinctus*)[1,2]

Charles K. Job, Rita M. Sanchez, and Robert C. Hastings[3]

The histopathological changes in the nine-banded armadillo (*Dasypus novemcinctus*) experimentally infected with *Mycobacterium leprae* have been described in detail by many authors ([1, 2, 7–9]). Lepromatous granulomas have been demonstrated in the skin, peripheral nerves, liver, spleen, lymph nodes, lung, heart, kidneys, adrenal glands, ovaries, and the intestines. Binford, *et al.* ([2]) in a study of 15 armadillos infected with *M. leprae* found infection of the brain in 3, of the meninges in 5, and of the spinal cord in 1.

In this paper, a detailed histopathological description of the appearance of the brain in lepromatous armadillos is given and its significance discussed.

MATERIALS AND METHODS

Brains removed in entirety from 10 lepromatous armadillos which had disseminated disease with lepromatous granulomas in the liver, spleen and lymph nodes were examined. Eight of them were experimentally infected with doses of *M. leprae* varying from 2.3×10^8 to 2.3×10^9 intravenously. Two animals had developed the infection in the wild and had evidence of lepromatous disease when captured. The livers and spleens of the animals were heavily infected, containing from 1.7×10^6 to 3.0×10^{10} *M. leprae* per gram of tissue. Soon after removal, the brains were fixed in 10% neutral Formalin for a period of over 4 weeks. Representative blocks of tissues were cut from the cerebrum, cerebellum, and midbrain from each of these specimens, processed, and embedded in paraffin. Sections of 5 μm were made and stained with hematoxylin and eosin and a modified Fite's stain ([5]). The sections stained for acid-fast bacilli (AFB) were carefully examined under an oil-immersion lens.

RESULTS

Grossly, none of the brain specimens examined showed any significant change. There were no signs of inflammation such as erythema, meningeal exudate or necrosis.

Histopathological examination showed cellular infiltration of varying degree of the meningeal tissues in all 10 specimens. There were focal collections of mononuclear cells composed of mainly lymphocytes, a few plasma cells and many macrophages in the meninges. On acid-fast stain the macrophages contained many intracellular bacilli (Fig. 1).

In two animals, one experimentally infected and the other having the infection from the wild, the sections from the cerebrum, cerebellum, and midbrain showed evidence of infiltration of the brain parenchyma with *M. leprae*. There were obvious changes in the capillaries of the brain which showed hyperplasia and hypertrophy of the endothelial cells. A few small blood vessels had perivascular cuffing (Fig. 2). Focal microgranuloma formation composed of lymphocytes, plasma cells, and macrophages was seen around some medium-sized blood vessels. There was also extravasation of red blood cells around small capillaries. A few ganglion cells showed marked satellitosis, and some of them were surrounded by three to four glial cells. There was no evidence of necrosis or glial nodules.

On acid-fast stain many of the swollen endothelial cells of the small capillaries were packed with AFB (Fig. 3). There were several astrocytes, oligodendroglial, and microglial cells showing intracellular AFB (Fig. 4). The epithelial cells of the choroid plexus

---

[1] Wayne M. Meyers, M.D., Ph.D., kindly served as Editor in regard to the submission, review, revision, and acceptance of this manuscript.

[2] Received for publication on 14 October 1987; accepted for publication in revised form on 12 February 1988.

[3] C. K. Job, B.Sc., M.D., F.R.C.Path., F.A.M.S., Chief, Pathology Research Department; R. M. Sanchez, B.Sc., Supervisory Microbiologist, Microbiology Research Department; R. C. Hastings, M.D., Ph.D., Chief, Laboratory Research Branch, GWL Hansen's Disease Center, Carville, Louisiana 70721, U.S.A.

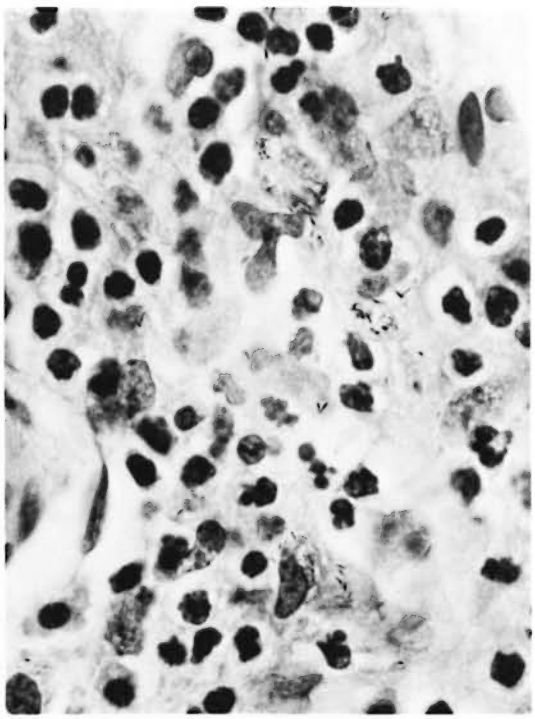

FIG. 1. Meninges infiltrated with lymphocytes and macrophages containing AFB (modified Fite ×700).

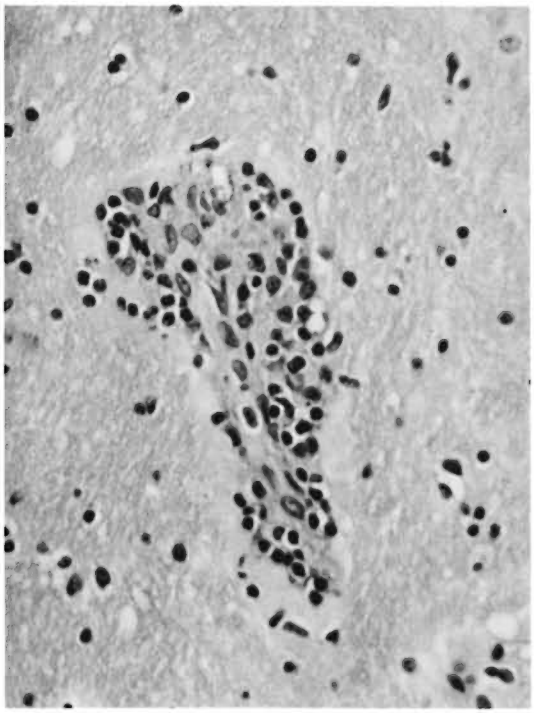

FIG. 2. Perivascular cuffing of mononuclear cells (H&E ×300).

were also invaded by *M. leprae*. The most interesting finding was the presence of AFB in a few degenerating cells which in all probability are neuronal cells in the midbrain, the cerebellum, and the cerebrum (Figs. 5 and 6).

DISCUSSION

Examination of the brains of these armadillos with disseminated leprosy consistently showed evidence of meningeal infection by *M. leprae* in all of the 10 animals examined. This finding is in agreement with an earlier report by Binford, *et al.* ([2]).

There is bacteremia in most if not all armadillos with disseminated leprosy. The finding of bacilli in blood is one of the tests used routinely in the laboratory for screening armadillos for disseminated disease ([8]). In two animals there was well marked parasitization by *M. leprae* of the numerous endothelial cells of the capillaries of the brain. Invasion of endothelial cells by *M. leprae* is fairly common in lepromatous disease and has been reported earlier ([4]). Some workers believe that the endothelial cells may even be preferential sites for proliferation of *M. leprae* ([10]). The penetration of the blood-brain barrier occurs following massive invasion of *M. leprae* into endothelial cells. The perivascular cuffing and the extravasation of red blood cells around capillaries and the infection of neuronal cells, astrocytes, and other glial cells by *M. leprae* provide clear evidence of the break in the blood-brain barrier. The organisms appeared to have readily multiplied in the neuronal cells and the glial cells that were infected. There was also infiltration by *M. leprae* of the epithelial cells of the choroid plexus and of the endothelial cells of the capillaries of the plexus.

Organisms which are considered neurotrophic, such as the rabies virus, parasitize and multiply preferentially in neuronal and neuroglial cells. In the present studies in the armadillo brain, *M. leprae* are found to parasitize and multiply in endothelial cells, macrophages, neuronal cells, and glial cells. The cells of the reticuloendothelial system of immunologically compromised mice are packed with *M. leprae* long before the nerves

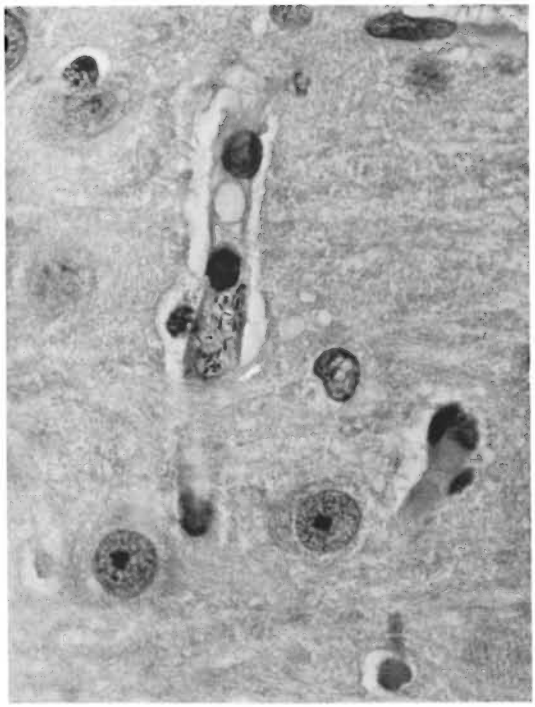

FIG. 3. Endothelial cell containing many AFB (modified Fite ×700).

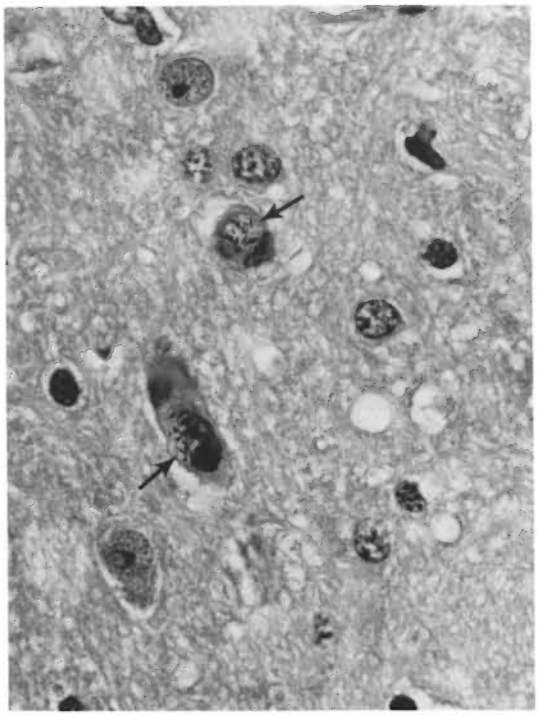

FIG. 4 A glial cell and an endothelial cell containing AFB (modified Fite ×700).

in the animal are even invaded ([6]). There may be a need to reassess the widely prevalent concept of the neurotropism of *M. leprae*. Schwann cells are invaded by *M. leprae* in all patients with leprosy to produce damage to peripheral nerves. It may be that the very obvious manifestations of nerve paralysis and the irreversible nature of the nerve damage produced by *M. leprae* are responsible a) for underestimating their invasion into other tissues, such as the cells of the reticuloendothelial system ([8]), liver cells ([7]), smooth and striated muscle cells, cartilage and bone cells ([6]), and b) for labeling *M. leprae* as neurotrophic. *M. leprae* certainly should be considered a neuropathic organism. However, the capacity of *M. leprae* to invade Schwann cells could well be incidental and not preferential even though the major symptoms and morbidity are localized at the peripheral nerves.

This is the first report known to the authors in the English literature with a clear demonstration of *M. leprae* inside the body of neuronal cells, although bacilli have been demonstrated earlier in the axons ([3]). Neurons are not phagocytes, and they are not known to ingest foreign particles. This study showing neuronal cells to contain *M. leprae* again emphasizes the fact that *M. leprae* are capable of invading and multiplying in any type of cell so long as the cell lives long enough and its physical environment is conducive to the growth of the organisms.

SUMMARY

The brains from 10 nine-banded armadillos with lepromatous leprosy were studied histopathologically. All of them showed evidence of lepromatous meningitis. In two there was invasion by *Mycobacterium leprae* into the brain tissue, with neuronal cells and glial cells containing intracellular bacilli. To our knowledge, this is the first report of meningoencephalitis in a lepromatous nine-banded armadillo.

RESUMEN

Los cerebros de 10 armadillos de 9 bandas con lepra lepromatosa se estudiaron histopatológicamente. Todos ellos mostraron evidencias de meningitis leprosa. En dos, hubo invasión por el *Mycobacterium leprae*

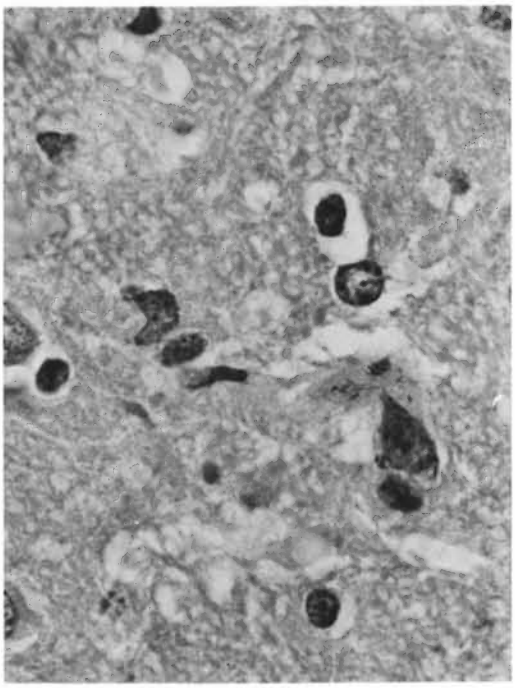

FIG. 5. A neuronal cell containing numerous AFB in the cytoplasm (modified Fite ×900).

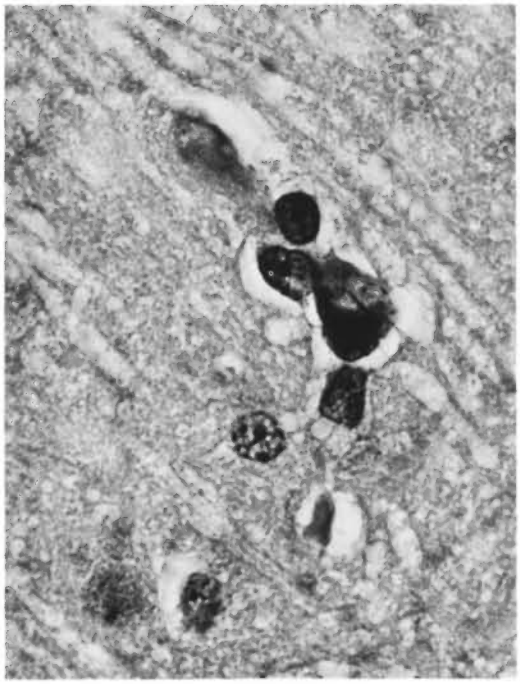

FIG. 6. Another degenerating neuronal cell with acid-fast organisms (modified Fite ×900).

del tejido cerebral con bacilos intracelulares en células neuronales y células gliales. Este es el primer reporte sobre meningo-encefalitis en armadillos de 9 bandas lepromatosos.

RÉSUMÉ

On a mené des études histopathologiques sur des cerveaux provenant de dix tatous à neuf bandes, atteints de lèpre lépromateuse. Tous les animaux présentaient des signes de méningite lépromateuse. Chez deux d'entre eux, on a observé une invasion du tissus cérébral par *Mycobacterium leprae*, avec présence de neurones et de cellules gliales contenant des bacilles intracellulaires. Pour autant que les auteurs en soient informés, ceci est le premier rapport d'une méningo-encéphalite chez un tatou à neuf bandes lépromateux.

Acknowledgments. This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Geneva, Switzerland, and the National Institutes of Allergy and Infectious Diseases, Inter-Agency Agreement 1-Y01-AI-5004-02. We are very thankful to Dr. James C. Harkin, Professor of Neuropathology, Tulane Medical Center, New Orleans, Louisiana, U.S.A., for his assistance in reviewing the slides. We would like to record our gratitude to Messrs. Joe L. Allen and Gregory T. McCormick for their excellent technical work and to Mrs. Mary M. Jackson for secretarial assistance.

REFERENCES

1. BINFORD, C. H., ISSAR, S. L., STORRS, E. G. and WALSH, G. P. Leprosy in the nine-banded armadillo (*Dasypus novemcinctus*); summary of post-mortem observations made on seven animals. Int. J. Lepr. **42** (1974) 123.
2. BINFORD, C. H., STORRS, E. E. and WALSH, G. P. Disseminated infection in the nine-banded armadillo (*Dasypus novemcinctus*) resulting from inoculation with *M. leprae*; observations made on 15 animals studied at autopsy. Int. J. Lepr. **44** (1976) 80–83.
3. BODDINGIUS, J. The occurrence of *M. leprae* within axons of peripheral nerves. Acta Neuropathol. (Berl.) **27** (1974) 257–270.
4. JOB, C. K. *Mycobacterium leprae* in nerve lesions in lepromatous leprosy; an electron microscopic study. Arch. Pathol. **89** (1970) 195–207.
5. JOB, C. K. and CHACKO, C. J. G. A modification of Fite's stain for demonstration of *M. leprae* in tissue sections. Indian J. Lepr. **58** (1986) 17–18.
6. JOB, C. K., CHACKO, C. J. G., VEREGHESE, R. and PADAM SINGH, S. Leproma of the mouse foot. Lepr. Rev. **46** (1975) 39–49.
7. JOB, C. K., KIRCHHEIMER, W. F. and SANCHEZ, R. M. Liver lesions in experimental lepromatous leprosy of the armadillo; a histopathologic study. Int. J. Lepr. **48** (1978) 1–8.
8. JOB, C. K., SANCHEZ, R. M. and HASTINGS, R. C. Manifestations of experimental leprosy in the ar-

madillo. Am. J. Trop. Med. Hyg. **34** (1985) 151–161.

9. KIRCHHEIMER, W. J., STORRS, E. E. and BINFORD, C. H. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. II. Histopathological and bacteriologic post-mortem findings in lepromatoid leprosy in the armadillo. Int. J. Lepr. **40** (1972) 229–242.
10. MUKHERJEE, A. and MEYERS, W. M. Endothelial cell bacillation in lepromatous leprosy; a case report. Lepr. Rev. **58** (1987) 419–424.