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Borderline Leprosy in an Experimentally Infected Armadillo[1]

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In 1971 and 1972, Kirchheimer and Storrs ([2,3]) and Kirchheimer, *et al.* ([4]) reported for the first time the transmission of leprosy to the nine-banded armadillo (*Dasypus novemcinctus* Linn.). The immunologically unaltered armadillo developed an experimental disease resembling lepromatous leprosy in man. It was further learned that under experimental conditions susceptibility to leprosy seems usual for the armadillo, unlike humans, and resistance to the disease is seen only in about 20% of the infected animals ([5]). It has also been reported that all animals which developed leprosy had the lepromatous type of the disease and those which were resistant did not show any evidence of leprosy.

In this paper we present the histopathological appearance of the lesions of an armadillo infected with *Mycobacterium leprae* which showed features characteristic of borderline leprosy.

MATERIALS AND METHODS

In one experiment six armadillos were infected intravenously with 4.66×10^8 *M. leprae* in 0.1 ml of saline. The bacilli were isolated from an experimentally infected armadillo. Four of these six infected animals developed disseminated lepromatous leprosy with a bacterial load of approximately 10^{10} per gram of lymph node tissue within a period of 12 months to 16 months. Three out of these four animals were sacrificed and one died. The present report concerns animal #5 of this series which showed macrophages containing acid-fast bacilli in tissue sections of ear clippings and was sacrificed 39 months after infection. Animal #6 of this series was also sacrificed a week later and did not show any evidence of disseminated leprosy.

The tissues from the liver, spleen, lymph node, ear, and sciatic nerve from the autopsied armadillo #5 were fixed in 10% formalin and processed for paraffin sections. Sections were cut at 5 μ thickness. One section was stained with hematoxylin-eosin stain. The other was stained with a modified Fite-Faraco stain in which, instead of 1% hydrochloric acid in 70% alcohol, 5% sulfuric acid was used for decolorizing, and in which 0.5% Harris' hematoxylin was used as the counterstain instead of 1% methylene blue solution.

RESULTS

The bacterial loads in the organs examined were 3.74×10^7 per gram of tissue in the liver, 1.08×10^8 in the spleen, and 5.69×10^8 in lymph nodes. The histopathological appearances of the ear, sciatic nerve, liver, spleen, and lymph nodes were as follows.

Ear. The epidermis showed no significant change. Between the epithelium and the cartilage, on both the inner and the outer aspects of the ear, there were small collections of inflammatory cells (Fig. 1), consisting of lymphocytes, epithelioid cells, and macrophages unevenly mixed together. The inflammatory infiltrate was pronounced in and around cutaneous nerve bundles. Some of the nerves were partially destroyed by the intraneural inflammation (Fig. 2). There were no foamy macrophages. This histopathological appearance was consistent with borderline tuberculoid leprosy. Acid-fast stain showed some bacilli inside Schwann cells, perineurial cells, and macrophages (Fig. 3).

Sciatic nerve. The nerve had nine fasciculi of which only three were involved in the infection. The uninvolved and the in-

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fected fasciculi remained side by side (Fig. 4). There was marked proliferation of the perineurial cells up to seven layers, producing thickening of the perineurium. The nerve parenchyma was infiltrated by macrophages, epithelioid cells, and lymphocytes (Fig. 5). Nerve destruction was seen in these areas of inflammation. It is interesting to note that these granulomatous lesions were focal even within one fasciculus. Foamy changes of macrophages were not observed.

Acid-fast stain showed bacilli only in the fasciculi with the inflammatory reactions. Organisms were present in perineurial cells, Schwann cells, and macrophages. The acid-fast bacilli were numerous in areas where the inflammatory infiltrate was concentrated.

The histopathological appearance of epithelioid cells and lymphocytes infiltrating focal areas in the nerve was consistent with borderline tuberculoid leprosy. However, the presence of numerous acid-fast bacilli in macrophages and Schwann cells was consistent with the tissue reaction being in a downgrading phase with the disease pro-

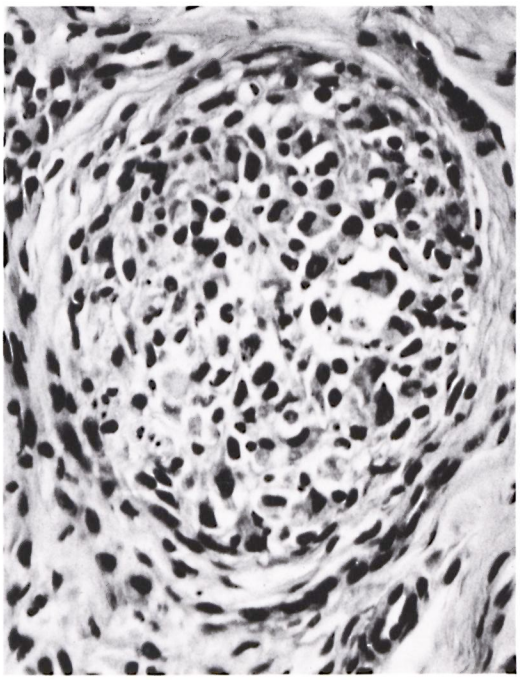

FIG. 2. A dermal nerve showing intraneural and perineural infiltration with mononuclear cells. (H & E ×600).

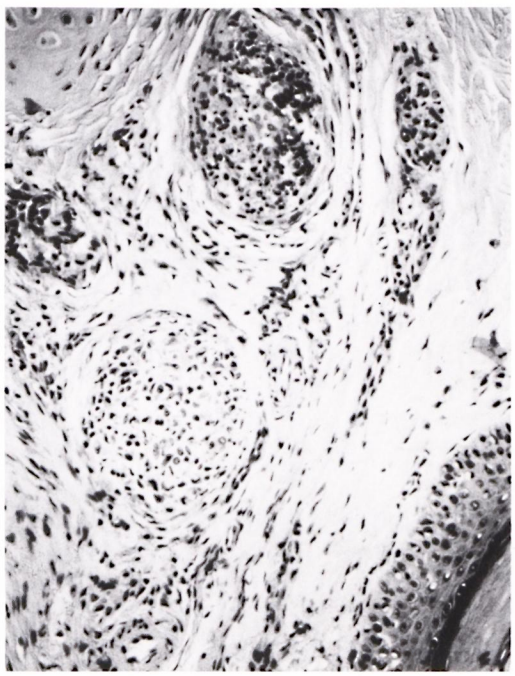

FIG. 1. Section of ear showing focal collections of mononuclear cells in the subepithelial tissue and in dermal nerves. (H & E ×300).

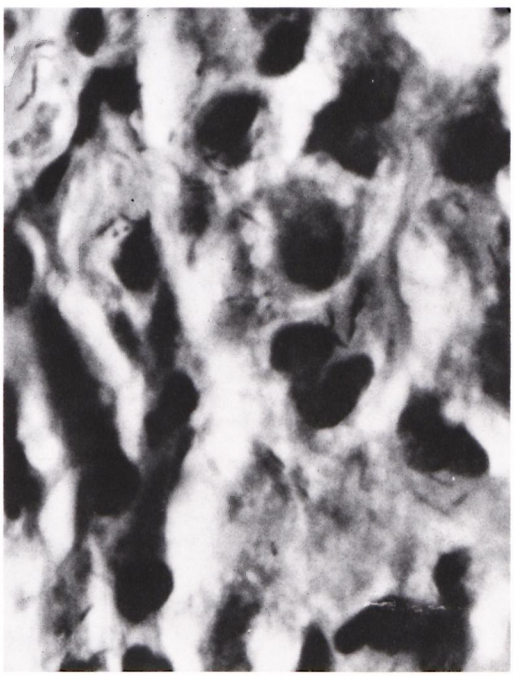

FIG. 3. Nerve showing a few acid-fast bacilli inside macrophages and Schwann cells. (A.F.B. ×1900).

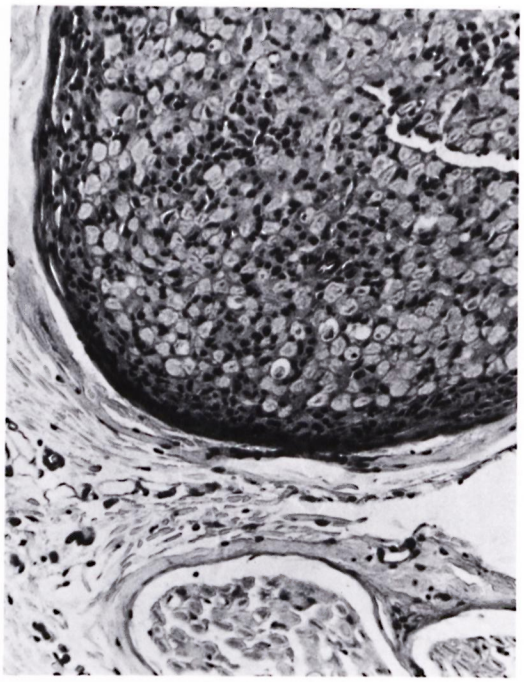

FIG. 4. Cross section of sciatic nerve to show infected and uninfected fasciculi side by side. The infected nerve bundle shows thickening of perineurial cells and focal intraneural collections of mononuclear cells. (H & E ×300).

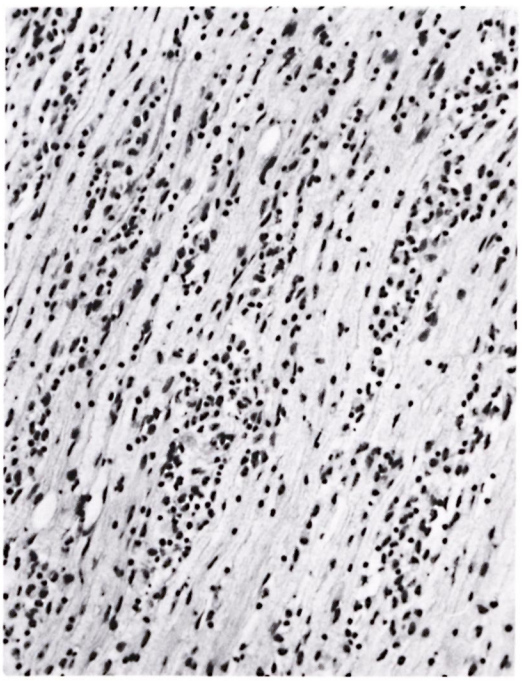

FIG. 5. Longitudinal section of a fasciculus of sciatic nerve. Numerous lymphocytes and macrophages infiltrate and, in areas, destroy the axons. (H & E ×300).

gressing towards the lepromatous side of the spectrum.

Liver. The architecture of the liver was preserved. Focal areas of inflammatory cell collections were seen throughout the liver tissue (Fig. 6). These microgranulomas had no definite relationship to the liver lobules. They were composed of lymphocytes, macrophages, and epithelioid cells. Foamy macrophages were absent (Fig. 7).

Acid-fast stain showed a few bacilli inside Kupffer's cells, liver cells, and many bacilli inside macrophages in the granuloma.

Spleen. The red pulp of the spleen showed the sinusoids dilated and filled with blood. The branches of the splenic artery were surrounded by small collections of lymphocytes. There were also microgranulomata, formed by aggregates of macrophages maturing to epithelioid cells, at the periphery of the lymphocyte collections and in focal areas in the red pulp. Acid-fast staining showed organisms inside macrophages in the granuloma, in some reticuloendothelial cells lining the sinusoids, and in smooth muscle cells of the trabeculae. Foamy macrophages were not seen.

Lymph node. The lymph node was enlarged. Large collections of what looked like epithelioid cells infiltrated the subcapsular regions and the site between the pyramidal areas, replacing the lymphocytes in the follicles and the medullary cords. A few giant cells were also present among them. Acid-fast staining showed most of the epithelioid cells to be free of bacilli. Some of these had organisms, varying in number from 1 to 14 per cell.

DISCUSSION

The histopathological appearance of lepromatous leprosy in armadillos has been well described in earlier papers ([1,4]). Lepromatous granulomata in the skin, liver, spleen, and lymph nodes consist of large collections of macrophages with foamy vacuolated cytoplasm. Acid-fast organisms fill these cells. The sciatic nerve shows perineurial collections of macrophages which also infiltrate the nerve parenchyma. Perineurial cells, Schwann cells, and macro-

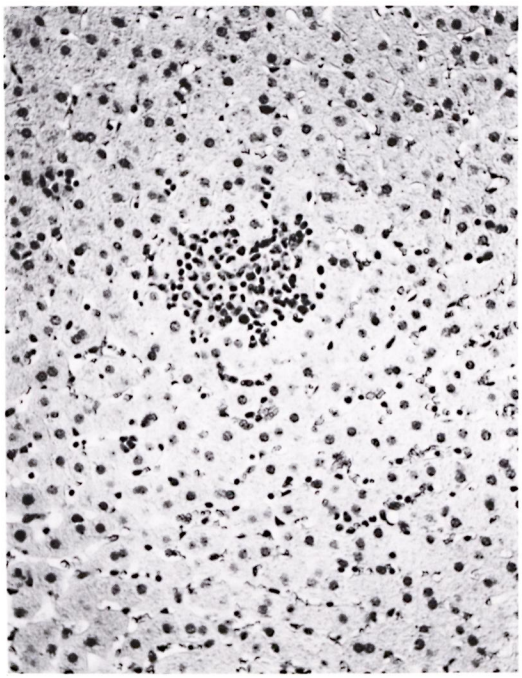

FIG. 6. Focal microgranuloma in liver tissue. (H & E ×300).

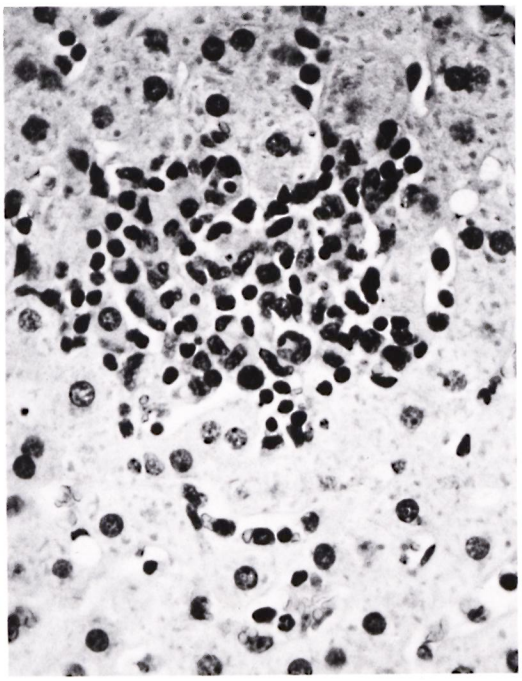

FIG. 7. The microgranuloma in liver is composed of an uneven mixture of lymphocytes, macrophages and epithelioid cells. (H & E ×600).

phages are packed with bacilli. Myelinated nerve fiber distribution is slight ([7]).

In the present armadillo, there were collections of epithelioid cells and lymphocytes in the skin, liver, spleen, and lymph nodes. Although there were macrophages containing bacilli, there were no foamy changes. The sciatic nerve lesion selectively involved only 3 out of 9 fasciculi, and in each involved fasciculus there were focal areas of inflammation and destruction. The cutaneous nerves showed marked intraneural infiltration by lymphocytes and macrophages, and destruction of nerve parenchyma. The load of acid-fast bacilli in these lesions was comparatively small. All these findings can fit into a histopathological picture of a borderline tuberculoid lesion undergoing a downgrading phase. It is clear from this study that armadillos can mount a delayed hypersensitivity tissue reaction to *M. leprae*, producing a granuloma composed of epithelioid cells and lymphocytes.

Whereas the four lepromatous animals had 10^{10} bacilli per gram of lymph node tissue within 10 months to 16 months, in this armadillo there were only 10^{8} bacilli per gram of lymph node tissue 39 months after the infection. The granulomata had many epithelioid cells which had apparently digested their bacillary content, accounting for the low bacillary load. However, the lesions also showed bacilli-filled macrophages and Schwann cells, and a comparatively small number of lymphocytes, suggesting a downgrading of the granuloma toward the lepromatous side of the spectrum. Presumably, if the animal's cell-mediated immunity broke down even further, lepromatous degeneration of the granuloma would follow.

In a study by Kirchheimer and Sanchez ([5]) it was shown that whatever the infecting dose of *M. leprae*, almost the same percentage of armadillos developed disseminated disease. However, the period taken for the development of the disseminated disease and for building up to a bacterial load of 10^{11} per gram of tissue varied. The animals infected with 10^{3} organisms took 650 days to 1285 days; whereas the animals receiving 10^{4} *M. leprae* took 421 days to 897 days to reach this high bacterial load. Thus, the infecting dose of *M. leprae* plays a role in the time required to develop

overtly disseminated disease. Another factor that could affect the time required to reach a given load of bacteria is the percentage of viable organisms in the inoculum. Within groups which receive the same dose of *M. leprae* containing the same number of live organisms, there is considerable variation (nearly twofold) in the time required to attain the same severity of infection.

The high susceptibility of nine-banded armadillos to leprosy may be a characteristic of the species, as pointed out by Kirchheimer and Sanchez ([5]). The variation in susceptibility among the susceptible animals is remarkable, however, and could be due to varying degrees of macrophage activation, differences in the cell-mediated immune responses, or other yet unknown factors. It is suggested that there are a certain number of susceptible armadillos which are capable, at least for a time, of producing an epithelioid cell granuloma and limiting the spread of the infection as was the case with the present armadillo. Delayed-type hypersensitivity reactions to antigens of *M. leprae* have been demonstrated by lepromin testing and by lymphocyte blast transformation studies in armadillos vaccinated with heat-killed leprosy bacilli suspended in Freund's incomplete adjuvent ([6]). The fact that this is the only infected armadillo reported as yet with delayed hypersensitivity tissue responses to *M. leprae* in its lesions should not minimize its role. A systematic study to assess the degree of cell-mediated immunity to *M. leprae* among susceptible armadillos might establish the frequency of this phenomenon among them.

It is possible that armadillos which develop disseminated disease fairly quickly following the infection may be comparable to humans with primary lepromatous leprosy, and armadillos that take a much longer time and pass through a borderline phase may be similar to humans developing secondary lepromatous disease.

SUMMARY

Out of six armadillos infected intravenously with 4.66×10^8 *M. leprae*, one developed a delayed hypersensitivity tissue response with the formation of an epithelioid cell granuloma. It is suggested that, although 80% of armadillos are susceptible to infection with *M. leprae*, some among these susceptible ones will exhibit varying degrees of delayed hypersensitivity reactions. These animals may go through a borderline phase before ultimately undergoing lepromatous degeneration.

RESUMEN

Uno de seis armadillos inoculados intravenosamente con 4.66×10^8 *M. leprae*, desarrolló una respuesta de hipersensibilidad retardada, granulomatosa y con células epitelioides. Se sugiere que, aunque el 80% de los armadillos son susceptibles a la infección con el *M. leprae*, algunos animales susceptibles pueden exhibir diferentes grados de hipersensibilidad retardada. Estos animales pueden atravezar por una fase intermedia antes de presentar la degeneración lepromatosa.

RÉSUMÉ

Parmi six armadillos infectés par voie intraveineuse par 4.66×10^8 *M. Leprae*, un a développé une réponse tissulaire d'hypersensibilité retardée, avec formation d'un granulome à cellules épithélioïdes. On suggère que, malgré le fait que 80% des armadillos soient susceptibles à l'infection par *M. Leprae*, certains parmi ces animaux susceptibles démontrent des différences dans le degré de réaction d'hypersensibilité retardée. Ces animaux peuvent passer par une phase dimorphe (borderline) avant de témoigner plus tard d'une dégénérescence lépromateuse.

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