Prevalence and Significance of Positive Mitsuda Reaction in the Nine-banded Armadillo (*Dasypus novemcinctus*)[1]

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Nine-banded armadillos have been found to be highly susceptible to leprosy ([9]). Up to 93% of experimentally infected animals develop the disease ([8]). Naturally acquired leprosy in armadillos was first reported in 1975 ([13]). Later it was found that indigenous leprosy existed among armadillos as early as 1961—long before the studies to experimentally infect the armadillos with *Mycobacterium leprae* were initiated in 1968 ([12]). The prevalence of wild leprosy among the animals has been variously estimated from 2% ([4]) to 6.8% ([10]). Where examination of animals was confined to specific sites in endemic areas, the prevalence was found to be as high as 30% ([10]).

The lepromin test assessed on the basis of the Mitsuda reaction has been a good indicator of the animal's susceptibility to infection with *M. leprae*. Mitsuda-positive armadillos are resistant, and the negative ones are susceptible ([5]). In this paper, we have studied the prevalence of lepromin positivity among armadillos with a view to understanding its significance.

MATERIAL AND METHODS

The armadillos in this study were captured from the wild in the state of Louisiana, U.S.A., and were admitted into the laboratory. On admission they were screened for indigenous leprosy ([6]). Of the 161 animals received from January to November 1986, 7 died before screening procedures were conducted and another 52 died within a month due to miscellaneous causes. The other 102 animals were lepromin tested. Standard lepromin containing 1.6×10^8 *M. leprae* per ml in suspension prepared from infected armadillo tissues was used ([7]); 0.1 ml of the suspension was injected intradermally into the abdominal skin, and the site of injection was identified with tattoo marks. At 21 days the skin reactions were assessed grossly. The induration of the skin in two directions perpendicular to each other was measured, and the mean reading in mm was recorded. All test sites were biopsied, and the tissues were fixed in 10% Formalin, and 5 μ sections were made and stained with hematoxylin and eosin and a modified Fite's stain ([3]).

RESULTS

Of the 154 animals screened for indigenous leprosy, 10 (6.5%) had the disease.

In five animals the induration of the lepromin test site at 21 days measured more than 5 mm, and in three the gross reading was 2 mm to 3 mm. In 94 there was no obvious reaction at the test site (Table 1).

Biopsies from all test sites were examined histopathologically, and a variable appearance ranging from a large, well-formed epithelioid cell granuloma to a few scattered mononuclear cells containing collections of acid-fast bacilli (AFB) in the dermis was recorded. In one animal there were a few lymphocytes and histiocytes, with no evidence of any acid-fast organisms. In this animal either the biopsy missed the injection site or the macrophages successfully removed or destroyed the organisms put into the site 21 days earlier.

According to the histopathological appearance, the lepromin reactions were classified as tuberculoid, borderline tuber-

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TABLE 1. *Gross readings of lepromin tests.*

Measurement	No.
5–14 mm	5
2–3 mm	3
Negative	94
Total screened	102

culoid, borderline lepromatous, and lepromatous reactions (Table 2).

Tuberculoid reaction. The granulomatous reaction was large, occupying nearly 75% to 90% of the dermis. Most of the cells were epithelioid cells. There were also many lymphocytes and a few Langhans giant cells. In 4 of the 5 there was a fairly large central area of necrosis, involving the collagen tissue. There were a few clumps of AFB extracellularly in the necrotic area and a few intracellular organisms in some macrophages immediately surrounding the necrotic area. Five animals showed this type of reaction, and all of them were grossly positive with ≥5 mm induration.

Borderline tuberculoid reaction. There was a well-marked granulomatous reaction occupying 40% to 50% of the dermis. The granuloma was composed of mainly epithelioid cells. Some lymphocytes and Langhans giant cells were also seen. In some areas there was intermingling of macrophages along with epithelioid cells, and the macrophages had intracellular AFB. No necrosis was present. Four animals were found to have this type of reaction and none of them was grossly positive.

Borderline lepromatous reaction. The granuloma occupied 10% to 15% of the dermis and was composed mainly of macrophages. A few epithelioid cells, scattered lymphocytes, and plasma cells were also present. Acid-fast staining showed clumps of intracellular bacilli. Eight animals had this type of reaction and none of them had a positive gross reading.

Lepromatous reaction. The amount of inflammatory cells at the lepromin site occupied less than 5% of the tissue. However the tissue reaction was varied. In 19, there were macrophages containing AFB distributed in a wide area in the dermis. There was very little attempt by the cells to collect together to form clumps. The macrophages were present mainly around blood vessels. In four, in addition to scattered macrophages containing AFB, there was a focal micronodular spot with a collection of spindle-shaped cells and a few foamy giant cells containing AFB. In the other 61, there were small focal collections of macrophages containing AFB. The majority of the cells in some biopsies had a pink granular cytoplasm, and in most of them the cell cytoplasm was foamy. Eighty-four animals showed lepromatous lepromin reactions. Three of these had 2-mm to 3-mm reactions grossly.

TABLE 2. *Histopathological classification of lepromin tests.*

Classification	No.	Lepromin reaction
Tuberculoid	5	Positive (8.8%)
Borderline tuberculoid	4	
Borderline lepromatous	8	Negative (91.2%)
Lepromatous	84	
Nonspecific reaction	1	
Total	102	100%

Tuberculoid and borderline tuberculoid reactions were considered Mitsuda positive (8.8%). Borderline lepromatous, lepromatous and nonspecific reactions were considered negative (91.2%) (Table 2).

DISCUSSION

Mitsuda reactions with a gross reading below 3 mm had a histopathological appearance of a lepromatous lepromin. Four borderline tuberculoid reactions which are significant with regard to disease susceptibility grossly showed negative reactions. Therefore, any study requiring an accurate assessment of Mitsuda reactions in the armadillo should have a histopathological examination.

The Mitsuda reactions in 91.2% of the animals were negative. A tuberculoid reaction with granulomatous inflammation followed by elimination of most of the killed bacteria which had been injected was seen in only 8.8% of the animals. The histology of the Mitsuda reactions showed a spectrum of histopathological responses with the two polar forms, namely, tuberculoid and lepromatous, and the intermediate manifestations of borderline lepromatous and bor-

derline tuberculoid, very similar to what is reported in humans. It has been shown in leprosy patients that the histopathology of the lepromin reaction mirrors the histopathological appearance of the disease ([11]). Therefore, it is reasonable to expect that armadillos are capable of developing tuberculoid leprosy lesions.

In an earlier study, repeated lepromin testing was done on 28 armadillos infected with *M. leprae*. One animal with an initial borderline lepromin developed a tuberculoid lepromin reaction in 12 months. However, animals with an initial lepromatous lepromin reaction never showed conversion to a tuberculoid state in a follow-up study of 30 months ([7]). In human patients with a negative or a weak lepromin reaction, repeated lepromin testing at monthly intervals up to 18 months did not significantly change the reaction ([2]). In lepromatous disease, whatever be the mechanism of causation of the defect in the cellular immune response to *M. leprae*, it never seems to recover. It has also been shown that lepromin-positive animals are resistant to the development of lepromatous disease and do not yield large numbers of *M. leprae*. Therefore, programs designed to use armadillos to produce *M. leprae* would do well to screen the animals with a lepromin test before utilizing them for this purpose. Lepromin testing prior to infection did not interfere with the susceptibility and the eventual yield of *M. leprae* from the infected animals ([7]).

In a human population highly endemic for leprosy, the proportion of lepromin positivity is as high as 98% ([1]). However, in the armadillo population in Louisiana, which is highly endemic for leprosy, lepromin positivity is only 8.8%. The disease is known to have existed in humans for several thousand years, and during succeeding generations there may have been a selection for lepromin-positive individuals. Armadillo leprosy, as far as we know, is of recent origin. It is a deadly disease among them, killing the animal in less than a year after the development of generalized disease. It is possible, therefore, that in the course of time the prevalence of lepromin positivity among armadillos will increase considerably by selection.

There may be several reasons why there is such a low prevalence of the disease with such high susceptibility. It is possible that the mode of transmission of the disease in the armadillo is such that only a small number get infected. The dose of infection in the wild may be so small that an animal after acquiring the infection may take 2 to 4 years to develop a generalized disease and, therefore, there may exist many infected animals which are not recognizable by the methods now used to measure prevalence. This is supported by serologic data suggesting that phenolic glycolipid-I IgM antibody prevalence rates can be as high as 20% ([12]). Further, once the animal develops manifest disease it dies quickly in 6 months to 1 year and is, thus, only relatively briefly available for sampling.

There are several candidate vaccines now available for the prevention of leprosy. Tuberculoid disease is self-healing and is easily treated. Vaccines, to be protective, should be effective against lepromatous disease. With the availability of an animal model such as the armadillo, which on experimental infection develops lepromatous disease in a great majority of the cases, it is advisable to test the efficacy of candidate vaccines in this animal. An initial screening of a vaccine's capability for lepromin conversion in armadillos may be useful.

SUMMARY

One hundred two armadillos captured from the wild were lepomin tested. Nine of them (8.8%) showed a positive Mitsuda reaction. The histopathological appearance of the reaction had a spectrum showing tuberculoid, borderline tuberculoid, borderline lepromatous, and lepromatous histology. It is possible that armadillos can develop all the different types of leprosy seen in humans. The armadillo is a good animal model to test protective vaccines against leprosy.

RESUMEN

Ciento dos armadillos capturados en su habitat natural fueron "probados" con lepromina. Nueve de ellos (8.8%) mostraron una reacción de Mitsuda positiva. La apariencia histopatológica de la reacción tuvo un espectro con histología tuberculoide, tuberculoide subpolar, lepromatosa subpolar, y lepromatosa. Es posible que los armadillos puedan desarrollar todos los diferentes tipos de lepra observados en los humanos. El

armadillo es un buen modelo animal para probar las vacunas protectoras contra la lepra.

RÉSUMÉ

On a soumis à une épreuve à la lépromine 102 tatous capturés dans la nature. Neuf d'entre eux (8,8%) ont présenté une réaction de Mitsuda positive. L'aspect histopathologique de la réaction présentait un spectre histologique couvrant les formes tuberculoides, borderline tuberculoide, borderline lépromateuse, et lépromateuse. Il est possible que les tatous puissent développer tous les types de la lèpre, tels qu'on les voit chez les hommes. Le tatou est un animal qui convient pour étudier l'effet protecteur des vaccins contre la lèpre.

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