Mitsuda-Negative, Resistant Nine-Banded Armadillos and Enhanced Mitsuda Response to Live *M. leprae*

TO THE EDITOR:

Antigens that elicit protective T-cell responses in leprosy are yet to be determined. It is stated that the antigens of live mycobacteria are far more immunogenic and, therefore, more effective in inducing acquired immunity than those of killed mycobacteria ([1]). Although in animal experiments killed *M. leprae* were found to be as potent as live BCG in producing protective immunity ([10]), results of large-scale studies in humans using killed *M. leprae* as vaccines were thoroughly unsatisfactory ([3]). Recent studies have shown that secreted antigens from actively metabolizing mycobacteria may enhance the induction of protective immunity ([9]). In this study, the tissue response to viable *M. leprae* was compared to that of heat-killed *M. leprae* in a selected group of lepromin-negative, yet resistant, nine-banded armadillos.

Nine-banded armadillos caught in the wild in areas nonenzootic for leprosy and found negative for the wild infection were routinely skin tested with lepromin-A ([8]). The Mitsuda reaction was classified histopathologically according to the criteria laid down in an earlier study as lepromatous (LL), borderline lepromatous (BL), borderline tuberculoid (BT) and tuberculoid (TT) ([8]), taking into account the granuloma fraction (GF), cellular content and the bacterial load. The armadillos which had an LL Mitsuda reaction (lepromin negative) were injected intravenously with 1×10^9 *M. leprae* for the specific purpose of propagating large numbers of *M. leprae* for use in investigative studies. The majority of these armadillos develop signs of leprosy infection within a year of inoculation and eventually succumb to the experimentally induced disease in about 18 months ([11]). However, as we reported before, even among these Mitsuda-negative animals there was a subset resistant to the disease ([7]). Six such animals which had failed to show signs of leprosy infection for more than 3 years after their experimental inoculation were available for the present study.

Standard lepromin containing suspensions of 1.6×10^8 heat-killed *M. leprae* per ml was prepared from the foot pads of infected nude mice. Suspensions of 1.6×10^8 live *M. leprae* were prepared from the same source. Intradermal injections with 0.1 ml of the two suspensions were made, one in either side of the abdomen of the six Mitsuda-negative, resistant armadillos. The tested sites were biopsied at 21–28 days. The biopsies were fixed in 10% neutral formalin, processed for paraffin sections, 5-μm sections were cut, stained with hematoxylin and eosin (H&E) and a modified Fite's stain for acid-fast bacilli (AFB) ([5]).

The H&E-stained sections were evaluated for the granuloma fraction (GF), which was expressed as a percentage of the dermal tissue replaced by the inflammatory cells. The cellular contents of the granulomas

THE TABLE. *Details of the study.*

Serial no.	Animal no.	Reaction to live *M. leprae*			Reaction to heat-killed *M. leprae*		
		GF[a] %	BI[b]+	Class.[c]	GF[a] %	BI[b]	Class.[c]
1	5 E 5	60	5	LL	5	5	LL
2	5 E 7	20	5	LL	5	5	LL
3	6 E 1	90	0	TT	80	0	TT
4	A 1304	10	4	BL	<5	3	BL
5	6 H 11	40	5	BL	<5	3	BL
6	6 H 53	30	5	LL	10	5	LL

[a] GF = Granuloma fraction.
[b] BI = Bacterial load.
[c] Class. = Classification.

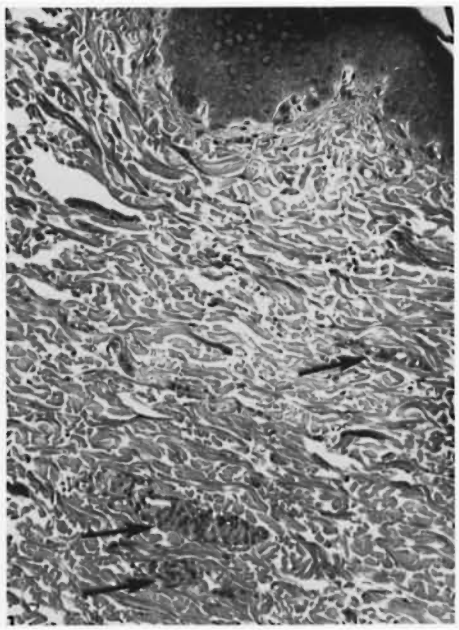

FIG. 1. Mitsuda reaction in a lepromin-negative armadillo intradermally injected with 1.6×10^7 killed *M. leprae*. Less than 5% of the dermis is replaced by microgranulomas. (H&E ×150).

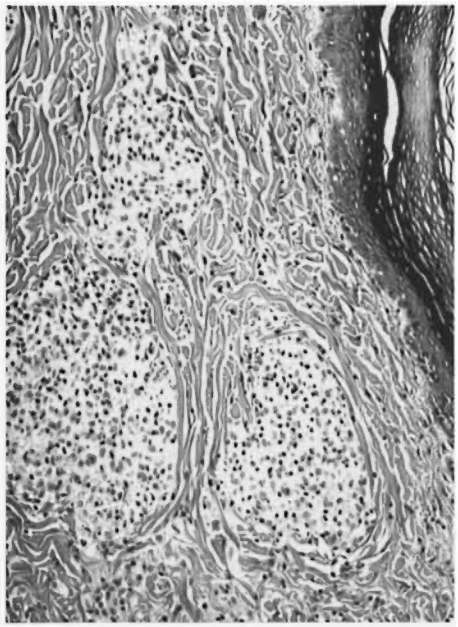

FIG. 2. Tissue response of same armadillo as in Figure 1 to 1.6×10^7 live *M. leprae* injected intradermally. Note a significant portion of the dermis is replaced by granuloma. (H&E ×150)

consisting of macrophages, epithelioid cells and lymphocytes were described. The sections stained for AFB were assessed for the bacillary load and were graded from 1 to 6+. To classify the response, only the cellular content and bacterial load were taken into account.

A summary of the results of the study is given in The Table. The bacterial load observed in the skin reaction to both live and heat-killed *M. leprae* was generally similar, and noted to differ in sections of only two animals. The granulomas of the Mitsuda reaction continued to remain in the lepromatous spectrum for all animals except one; animal #3 had developed a TT Mitsuda reaction during the course of infection. However, the other five animals, although unable to produce a tuberculoid granulomatous response to *M. leprae,* were resistant to the disease. It is thought that a positive Mitsuda reaction with well-organized epithelioid cells and lymphocytes demonstrates the presence of cell-mediated immunity and resistance to the disease ([2, 6]). From this study, it appears that the Mitsuda reaction provides information about the ability of the animal to produce a granulomatous response to the antigens of dead *M. leprae* but not necessarily about the killing of *M. leprae* and the animal's resistance to the infection. Could it be that the mechanism involved in the killing of mycobacteria may be different from that of the mechanism producing a granuloma? How else can the existence of animals resistant to the disease but unable to produce a granulomatous reaction to the antigens of *M. leprae* be explained?

It was also seen that the granulomas produced by the live organisms were consistently and significantly larger in size compared to those caused by killed *M. leprae* (Figs. 1 and 2). Both Mitsuda-negative and -positive animals showed augmented responses to live *M. leprae*. The magnitude of the difference in the Mitsuda-negative animals varied from 2 to 12 times larger in size at the live *M. leprae* site than at the lepromin site. The composition of the granulomas of all five Mitsuda-negative animals consisted of mostly macrophages, a few

lymphocytes and occasional plasma cells, and the bacillary load inside the macrophages did not show any significant difference. Similarly, there was no qualitative difference in the tuberculoid response of the one Mitsuda-positive animal in which the organisms were walled off along with a central area of necrosis surrounded by organized epithelioid cells and lymphocytes.

Secreted antigens of actively metabolizing live mycobacteria that are absent in dead ones may offer an explanation for this difference ([4]). The other possible explanation may be that the live *M. leprae* multiplied and caused an increase in the number of macrophages at the inoculation site. Further studies to explore the mechanism of resistance in lepromin-negative animals are underway.

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