

Comparison in Leprosy Patients of Fernandez and Mitsuda Reactions Using Human and Armadillo Antigens A Double-Blind Study^{1,2}

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The objective of this study was to determine the effectiveness of leprosy bacilli harvested from lepromas of infected armadillos when used as the antigen in the human lepromin test. This was done by comparing the delayed hypersensitivity skin reactions to two types of lepromin; that prepared from leprosy bacilli from lepromas of human leprosy patients and leprosy bacilli from lepromas of armadillos infected with human leprosy bacilli.

Since 1919 when Kensuke Mitsuda developed the prototype of lepromin as it is usually made, there has been no standardization of lepromin because of the difficulty in obtaining sufficient quantities of the leprosy bacilli⁽²⁾. However, with the development of the nine-banded armadillo as the first natural animal model to develop disseminated leprosy following injection with *Mycobacterium leprae* isolated from human tissues⁽³⁾, a source of sufficient bacilli in large quantities is now available for this standardized lepromin. A total of 1,255 gm of lepromas which contained an estimated 20 gm of leprosy bacilli was obtained from the first 13 infected armadillos and is indicative of the large quantities now available. It becomes necessary then to demonstrate that these bacilli can be effectively used in the lepromin test for humans and to establish some baseline findings of the type of skin reaction to expect. A comparative study of the reactions to human and armadillo lepromin in leprosy patients has been done by Meyers

*et al*⁴ in Zaire and the findings as presented in Sao Paulo, Brazil, September 1974 are similar to the results of this study⁽¹⁾.

MATERIALS AND METHODS

Preparation of lepromin. A double-blind study was used so that neither the investigators nor the people being tested were aware of which type of lepromin was being used. Two vials, each containing 30 cc of lepromin, were prepared at the National Institute of Dermatology in Caracas, Venezuela. The vials were coded "A" and "B" and the key to the code was unknown to the investigators. At the termination of the project, it was revealed that "A" contained a bacterial content of 160×10^6 bacilli per ml obtained from infected armadillos and vial "B" contained 160×10^6 bacilli per ml obtained from human lepromas. The human lepromin was prepared from cutaneous material obtained from Venezuelan cases of untreated lepromatous leprosy. The armadillo lepromin antigen was obtained from an armadillo (*Dasypus novemcinctus*) which is identified as Number 5 and is from Gulf South Research Institute, New Iberia, Louisiana. This armadillo 30 months before had been inoculated with material from a human lepromatous case from Paramaribo, Surinam. Neither of these antigens contained preservatives.

Patient population. One hundred and twelve patients of the Mahaica Hospital, the government hospital for leprosy patients of Guyana, South America volunteered to participate in the study. As based on the previously recorded clinical histories, the patient population consisted of 5 borderline, 1 indeterminate, 67 lepromatous, and 39 tuberculoid cases. The patients ranged in ages from 19 to 99 years. The distribution by age, sex and race is indicated in Tables 1 and 2. The Morphologic and Bacteriologic Indices of the patients are recorded in Table 3 and the duration of their disease in Table 4.

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TABLE 1. Age, sex and race distribution of leprosy patients.

Type	Sex		Race					Total
	F	M	A	I	E	O	M	
B	2	3	2	1	—	—	2	5
T	15	24	19	16	2	—	2	39
L	22	45	41	20	2	2	2	67
I	1	—	—	—	—	—	1	1
Total	40	72	62	37	4	2	7	112

Key: A = African, I = East Indian, E = European, O = Oriental, M = Mixed.

TABLE 2. Age of patient population at time of lepromin testing.

Age (years)	Type				Race					Total
	B	T	L	I	A	I	E	O	M	
less than 30	1	1	5	—	3	4	—	—	—	7
31-39	1	4	7	—	5	5	—	—	2	12
40-49	—	8	12	—	7	9	3	—	1	20
50-59	2	8	16	—	15	8	1	1	1	26
60-69	—	11	15	—	18	7	—	—	1	26
70-79	1	5	9	1	9	4	—	1	2	16
>79	—	2	3	—	5	—	—	—	—	5
Total	5	39	67	1	62	37	4	2	7	112

Key: A = African, I = East Indian, E = European, O = Oriental, M = Mixed.

TABLE 3. Bacteriologic and Morphologic Indices of patient population.

Type	BI		MI	
	Positive	Negative	Positive	Negative
B	1	4	—	5
T	1	38	—	39
L	30	37	5	62
I	—	1	—	1
Total	32	80	5	107

TABLE 4. Duration of disease before time of testing.

Years	B	T	L	I	Total
<1	—	—	—	—	—
1-5	1	—	6	—	7
6-10	2	3	5	—	10
11-20	1	9	12	—	22
>21	1	27	44	1	73
Total	5	39	67	1	112

Method used. Using a tuberculin syringe of 1 ml capacity, 0.1 ml of lepromin "A" was introduced intradermally into the volar surface of the right forearm, and at the same sitting, 0.1 ml of lepromin "B" was likewise injected intradermally into the volar surface of the left forearm of the same patient. A separate syringe and needle was used for each injection. The introduction of both types of lepromin into the same patient automatically eliminated any variables which could otherwise invalidate the study. All controls were essentially biologically inherent within each patient. Forty-eight hours after injection the Fernandez reaction was read for each arm, and the Mitsuda reaction was read 21 to 28 days later. Photographs were taken of the positive reactions and biopsies obtained from the intradermal site of reaction of both lepromin "A" and lepromin "B" in five patients. These seven millimeter punch biopsies were read by Dr. C. H. Binford, Armed Forces Institute of Pathology, Washington, D.C.

The criteria for determining positivity of the lepromin reaction was based on that of the WHO Report of Expert Committee meeting in Rio de Janeiro, 1953, and is as follows:

- Negative (-) lack of evident response or slight induration, without definite appearance of activity and less than 3 mm in diameter.
- Doubtful (\pm) induration measuring 3 to 4 mm in diameter.
- Positive (+) reactions with induration greater than 4 mm and less than 7 mm in diameter.
- (2+) reactions with induration greater than 7 mm and less than 10 mm in diameter.
- (3+) reactions with ulceration, or those with diameters greater than 10 mm but without ulceration.

All readings of both the Fernandez and Mitsuda reactions were done by the same investigator.

RESULTS

After decoding we designated lepromin "A" from armadillo lepromin as A and lepromin "B" from human as lepromin H. Table 5 summarizes the Mitsuda and Fernandez reactions for the total study population and demonstrates that the armadillo antigen

TABLE 5. *Reactions—total population.*

Reaction	Mitsuda		Fernandez	
	Right arm (armadillo)	Left arm (human)	Right arm (armadillo)	Left arm (human)
(-) Negative	34 (30.5%)	39 (34.8%)	51 (45.5%)	72 (64.2%)
(\pm) 3-4 mm	5 (4.4%)	10 (8.9%)	43 (38.3%)	27 (24.1%)
1+ >4 and <7	9 (8.0%)	12 (10.7%)	13 (11.6%)	5 (4.4%)
2+ >7 and <10	42 (37.5%)	31 (27.6%)	1 (0.8%)	1 (0.8%)
3+ >10 or with ulcerations	22 (19.6%)	20 (17.8%)	4 (3.5%)	7 (6.2%)
Total	112	112	112	112

TABLE 6. *Reactions in lepromatous cases.*

Reaction	Mitsuda		Fernandez	
	Right arm (armadillo)	Left arm (human)	Right arm (armadillo)	Left arm (human)
(-) Negative	31	32	32	45
(\pm) Doubtful (3-4 mm)	3	9	28	18
1+ >4 and <7	4	5	—	3
2+ >7 and <10	16	12	7	—
3+ >10 or with ulcer	13	9	—	1
Total	67	67	67	67

gives a positive as well as a negative reaction in the early Fernandez reading as well as in the delayed Mitsuda reaction. There was only a 4.8% difference in the negative reaction (Mitsuda test) as a total of 34 patients (30.3%) reacted negatively to the armadillo lepromin as compared to 39 patients (34.8%) negative reactions to the human lepromin. The positive reaction showed only slight variance for each degree of positivity: doubtful reaction (\pm), 5 patients; (1+) 3 patients; (2+) 11 patients; (3+) 2 patients.

The reduction of delayed hypersensitivity type of immunity known to exist in patients with lepromatous leprosy and formerly demonstrated by a negative Mitsuda reaction using human bacilli, is comparably demonstrated by using armadillo bacilli as seen in Table 6; 32 lepromatous cases reacted completely negative to human lepromin, i.e., absolutely no induration present, as compared to 31 cases which reacted completely negative to armadillo lepromin. In no cases of negative or positive reaction did the armadillo lepromin cause a different reaction than that seen using the human lepromin.

Table 7 illustrates the delayed hypersensitivity response known to exist in the Mitsuda

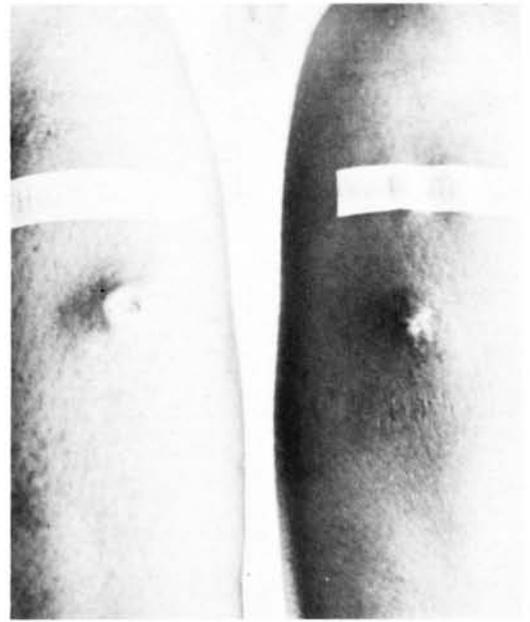


FIG. 1. Mitsuda reaction to armadillo antigen (right arm—left side of picture) and human lepromin in a 63-year-old tuberculoid leprosy patient. Note the induration and ulceration on both arms. Armadillo lepromin response: induration 22 mm, ulceration 8 mm. Human lepromin response: induration 20 mm, ulceration 7 mm.

TABLE 7. Reactions in tuberculoid cases.

Reaction	Mitsuda		Fernandez	
	Right arm (armadillo)	Left arm (human)	Right arm (armadillo)	Left arm (human)
(-) Negative	1	4	17	25
(\pm) Doubtful (3-4 mm)	1	1	12	7
1+ >4 and <7	3	3	6	1
2+ >7 and <10	8	10	—	1
3+ >10 or with ulceration	26	21	4	5
Total	39	39	39	39

TABLE 8. Reactions in borderline cases.

Reaction	Mitsuda		Fernandez	
	Right arm (armadillo)	Left arm (human)	Right arm (armadillo)	Left arm (human)
(-) Negative	2	3	1	2
(\pm) Doubtful (3-4 mm)	1	—	3	2
1+ >4 and <7	—	—	—	—
2+ >7 and <10	—	—	—	1
3+ >10 or with ulceration	2	2	1	—
Total	5	5	5	5

TABLE 9. *Reactions in indeterminate cases.*

Reaction	Mitsuda		Fernandez	
	Right arm (armadillo)	Left arm (human)	Right arm (armadillo)	Left arm (human)
(-) Negative	—	—	1	—
(±) Doubtful (3-4 mm)	1	—	—	—
1+ >4 and <7	—	1	—	1
2+ >7 and <10	—	—	—	—
3+ >10 or with ulceration	—	—	—	—
Total	1	1	1	1

TABLE 10. *Burning reaction at site of injection.*

	Lepromatous		Borderline		Tuberculoid		Indeterminate	
	armadillo	human	armadillo	human	armadillo	human	armadillo	human
Fernandez Rx								
0 Yes	11 (16.4%)	7 (10.4%)	0	0	7 (17.9%)	8 (20.5%)	0	0
1 No	56 (83.5%)	60 (89.5%)	5 (100%)	5 (100%)	32 (82.0%)	31 (79.4%)	1 (100%)	1 (100%)
Mitsuda Rx								
0 Yes	1 (1.4%)	1 (1.4%)	0	0	0	0	0	0
1 No	66 (98.5%)	66 (98.5%)	5 (100%)	5 (100%)	39 (100%)	39 (100%)	1 (100%)	1 (100%)

reaction in the tuberculoid cases. Figure 1 demonstrates the Mitsuda reaction to both the armadillo and the human lepromin. The negative response to the human lepromin in four patients and one doubtful reaction in another patient remained constant to both the armadillo and the human lepromin. The positive reactions in the remaining tuberculoid cases showed marked consistency, as five patients showed a (1+) response to both types of lepromin; only two patients differed in a (2+) response and five patients differed in the (3+) response.

The reactions of the five borderline cases and the one indeterminate case are illustrated in Tables 8 and 9, respectively.

Sixteen percent of lepromatous patients reported a burning sensation at the site of injection of the armadillo lepromin as compared to 10% of lepromatous patients experiencing the same reaction to the human lepromin (Table 10). Seventeen percent of tuberculoid patients had a similar reaction to the armadillo lepromin as compared to 20% of tuberculoid patients having a burning reaction to the human lepromin. This burn-

ing reaction was noted only at the time of the Fernandez reading, whereas only one patient of the total 112 patients complained of the reaction at the time of the Mitsuda reading. The borderline and indeterminate cases did not experience a burning sensation.

As illustrated in Table 11, 43% of lepromatous patients showed absolutely no difference in the measurement of the induration reaction to the armadillo and the human lepromin, whereas 49% showed a larger induration in the Mitsuda reaction to the armadillo antigen. Six cases (8%) had a 6 mm smaller Mitsuda reaction to the armadillo lepromin.

Of the tuberculin cases (Table 11) six cases (15%) had no difference in the measurement of the induration reaction to the armadillo and human Mitsuda tests; 23 patients (58%) had a larger induration response to the armadillo lepromin, whereas 10 cases (27%) had a smaller induration reaction to the armadillo lepromin.

In summary, 36 cases (32.3%) of the total 112 cases demonstrated no difference in the induration stimulated by the armadillo antigen and the human antigen. Fifty-nine cases

TABLE 11. Differences in induration measurements—armadillo vs human lepromin.

Mitsuda Reaction		Fernandez Reaction	
No. cases	Diameter of induration in armadillo reaction	No. cases	Diameter of induration in armadillo reaction
Lepromatous cases			
28	no difference	19	no difference
7	1 mm larger	11	1 mm larger
8	2 " "	11	2 " "
5	3 " "	5	3 " "
6	4 " "	3	4 " "
6	5 " "	1	5 " "
1	8 " "	1	6 " "
6	6 " smaller	6	1 " smaller
		5	2 " "
		1	3 " "
		3	4 " "
		1	14 " "
Tuberculoid cases			
6	no difference	13	no difference
2	1 mm larger	5	1 mm larger
6	2 " "	7	2 " "
3	3 " "	4	3 " "
2	4 " "	1	4 " "
6	5 " "	1	5 " "
1	6 " "	1	6 " "
3	8 " "		
1	1 " smaller	1	1 " smaller
3	2 " "	1	2 " "
4	3 " "	1	3 " "
2	4 " "	2	8 " "
		1	14 " "
		1	30 " "
Borderline cases			
2	no difference	1	no difference
2	2 mm larger	2	1 mm larger
1	5 " "	1	3 " "
		1	8 " "
Indeterminate cases			
1	0.1 mm smaller	1	18 mm smaller

(52.7%) showed a larger induration from the armadillo antigen, whereas 17 cases (15%) showed a smaller reaction.

Biopsy results. The biopsy specimens were evaluated using hematoxylin-eosin and Fite-Faraco stained sections. The histologic examination of the positive lepromin reaction to armadillo antigen revealed intensive infiltration by lymphocytes and histiocytes with epithelioid cells and giant cells. No bacilli were observed (Fig. 2). The reaction on the other arm of the same patient, where human lepromin had been given intradermally was

similar. The biopsy readings of three other positive lepromin readings showed cytologically similar reactions but in each instance the armadillo antigen stimulated a more severe reaction (Fig. 3). In each case, no bacilli were observed.

The histologic appearance of the negative lepromin reaction was marked by foamy histiocytes and, in several fields, well defined bacilli in phagocytes. The appearance of the biopsy specimens from the armadillo and the human lepromin tests were similar.

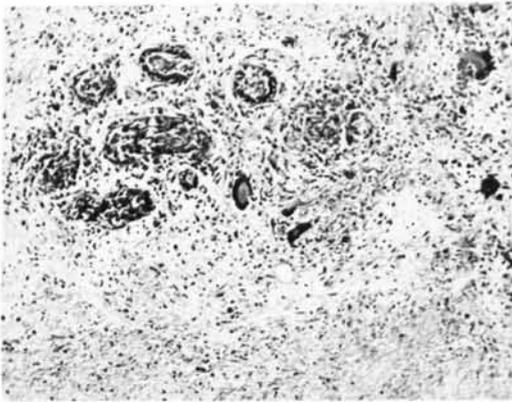


FIG. 2. Mitsuda reaction to human antigen in a 42-year-old European male tuberculoid patient. Observe the lymphocytic and histiocytic infiltration and the giant cell reaction. H&E stain, X160. AFIP Photo No. 75-764.

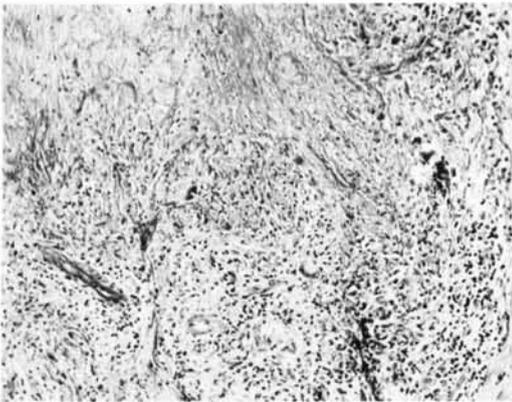


FIG. 3. Mitsuda reaction to armadillo antigen in same patient as Figure 2. Note the increased severity of lymphocytic and histiocytic reaction and the area of necrosis. H&E stain, X160. AFIP Photo No. 75-763.

DISCUSSION

The over-all results of this study indicate that in each patient tested the lepromin prepared from the lesions of infected armadillos stimulate a delayed hypersensitivity reaction similar to that initiated by lepromin prepared from the lesions of infected patients. Only three tuberculoid and two lepromatous patients responded to the one type of antigen and not to the other type. The probability of technical error must be considered when one evaluates this difference.

As previously indicated, in 32.3% of patients tested there was no difference in the

size of the induration reaction but in 52.7% there was a larger reaction to the armadillo lepromin. This would lead one to anticipate a large induration resulting from the armadillo lepromin if it becomes commercially available. However, it must be remembered that 15% of the cases actually had a smaller reaction to the armadillo lepromin.

The armadillo lepromin was consistent in producing a positive Mitsuda reaction in tuberculoid cases except in two patients as compared to five tuberculoid patients in whom the human lepromin produced a negative Mitsuda. The classification of these patients was based on the previously recorded histories and clinical typing of the patients, and not on the judgment of the investigators.

SUMMARY

The Mitsuda and Fernandez reactions to bacilli prepared from lesions of human patients and bacilli from infected armadillos were studied in 112 leprosy patients.

It was observed that there is no substantial difference when armadillo lepromin is used instead of human lepromin except that a larger induration occurs with the use of the armadillo lepromin in a majority of cases. The number of those reporting a burning sensation at the armadillo lepromin injection site was slightly higher in lepromatous patients; whereas the burning sensation was observed at the human lepromin injection site by the tuberculoid cases.

With the significant information compiled in this study, it is concluded that lepromin prepared from bacilli obtained from infected armadillos is as effective as that prepared from bacilli obtained from the lesions of leprosy patients.

RESUMEN

Se estudiaron las reacciones de Mitsuda y de Fernández a bacilos preparados de lesiones de pacientes humanos y bacilos obtenidos de armadillos infectados, en 112 pacientes con lepra.

Se observó que no había diferencias importantes cuando se utilizaba lepromina de armadillo en vez de lepromina humana, excepto que se observaba una induración mayor con la lepromina de armadillo en la mayoría de los casos. El número de pacientes que informaron sentir una sensación de quemadura fué ligeramente mayor en el sitio de inyección de lepromina de armadillo en pacientes lepromatosos; mientras que la sensación de quemadura fué observada en el sitio de

inyección de la lepromina humana en los pacientes tuberculoides.

Con la información significativa que hemos obtenido a través de este estudio, se concluye que la lepromina preparada con bacilos obtenidos de armadillos infectados es tan efectiva como la preparada con bacilos obtenidos de lesiones de pacientes con lepra.

RÉSUMÉ

Chez 112 malades de la lèpre, on a comparé des réactions de Mitsuda et de Fernandez à des préparations bacillaires obtenues à partir de lésions humaines ou d'armadillos infectés.

Il n'a pas été observé de différences notables entre des réactions obtenues par la lépromine d'armadillos et par la lépromine humaine, si ce n'est qu'une induration de plus grande dimension est observée à la suite de l'utilisation des lépromines d'armadillos dans la majorité des cas. Le nombre des malades rapportant une sensation de brûlure à l'endroit d'injection de la lépromine d'armadillo était légèrement plus élevé chez les malades lépromateux. Par contre, cette sensation de brûlure a été observée à l'endroit d'injection de lépromine humaine chez les malades tuberculoides. L'information fort significative recueillie à la suite de cette étude permet de conclure que la lépromine préparée à partir de bacilles obtenus d'armadillos infectés est aussi effective que celle que l'on obtient en utilisant des bacilles recueillis dans des lésions de lèpre humaine.

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