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The Lepromin Test in Rhesus Monkeys[1]

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A skin reaction to heated suspensions of lepromas was reported by Hayashi in 1918 ([5]). Mitsuda, in 1919, referred to the reports of Hayashi and earlier leprologists who had employed antigens obtained from nodules from leprosy patients as diagnostic skin tests in his comprehensive study of skin testing in 403 patients ([8]). Mitsuda's method of antigen preparation and grading of results, with some refinements, has come into general use in clinical and experimental leprology. Positive reactions are of two types: an early reaction at 1 to 2 days (Fernandez reaction) ([4]), and a late reaction which is maximal at 3 to 4 weeks (Mitsuda reaction). Histologically, the early reaction is characterized by acute inflammation and is analogous to the tuberculin reaction. The late reaction consists of infiltrates of epithelioid cells, lymphocytes, and a variable number of giant cells, and may or may not include necrosis and ulceration. A positive Mitsuda reaction is thought to reflect a pre-existing or induced cell-mediated immunity (CMI) against *Mycobacterium leprae.* The test site in nonreactive individuals consists of a histiocytic infiltrate ([9]).

Many investigators consider the lepromin test useful in assessing susceptibility to anergic forms of leprosy and for classifying the type of disease in leprosy patients: those with lepromatous leprosy are negative, while those with tuberculoid disease are positive. Most normal people, in both endemic and nonendemic areas, are Mitsuda positive ([9]). Other investigators have observed that normal monkeys are lepromin negative, although some become positive after inoculation with viable or killed *M. leprae* ([1, 2]).

We are currently developing animal models of leprosy in three species of nonhuman primates ([11]) and have evaluated the lepromin test in monkeys. We studied the type and dosage of lepromin most effective in eliciting a reaction in rhesus monkeys and characterized histologically the reactions that developed to ascertain whether positive or negative reactions bore any relationship to the status of the disease in experimentally inoculated animals.

MATERIALS AND METHODS

We used male or female rhesus monkeys (*Macaca mulatta*) born at the Delta Regional Primate Research Center, Covington, Louisiana, U.S.A., in an outdoor breeding colony. Once assigned to this project, the animals were individually caged in an indoor isolation facility, fed a standard laboratory ration, and given water *ad libitum.* All animals were tuberculin tested every 6 months and were negative. All manipulations (examinations, inoculations, etc.) were conducted with the monkeys under anesthesia with ketamine HCl. Intracutaneous inoculations were made into sites previously delineated by tattoos.

Details of the inoculations with viable *M. leprae* are shown in Table 1. Inoculations of viable human- or mangabey-derived *M. leprae* were made intravenously and/or intracutaneously using different numbers of bacilli (Table 1). Intracutaneous inoculations were distributed at multiple sites on the face, ears, and distal parts of the extremities.

Mangabey-derived *M. leprae* were obtained from biopsies of cutaneous lepromas from naturally or experimentally infected sooty mangabey monkeys (*Cercocebus atys*). The lepromas were homogenized and dif-

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TABLE 1. *Animal inoculation data; viable* M. leprae.

Animal no.	Sex	Source of inoculum[a]	Date inoculated	Dose of AFB		Status[b]
				i.v.	i.d.	
A125	M	M	12/80	1.5×10^8	6×10^8	L
A491	F	M	12/80	—	6×10^8	R
B465	M	H	6/82	5×10^{10}	5×10^{10}	L
B245	F	H	6/82	5×10^{10}	5×10^{10}	R
B347	M	H	6/82	5×10^9	5×10^{10}	R
A766	F	H	6/82	5×10^9	5×10^{10}	R
B539	M	H	6/82	5×10^8	5×10^{10}	I
B630	F	H	6/82	5×10^8	5×10^{10}	R
B685	M	H	6/82	5×10^7	5×10^{10}	R
B614	F	H	6/82	5×10^7	5×10^{10}	R
B960	F	H	6/82	—	5×10^{10}	R
B784	F	H	6/82	—	5×10^{10}	R
B849	M	H	6/82	5×10^{10}	—	R
A749	F	H	6/82	5×10^{10}	—	I
8664	M	M	11/82	5.4×10^8	5.4×10^8	L
B988	M	M	11/82	5.4×10^8	5.4×10^8	L
B845	F	M	11/82	5.4×10^8	5.4×10^8	R
B748	F	M	11/82	5.4×10^8	5.4×10^8	R
B244	M	—	—	—	—	C
9101	F	—	—	—	—	C
9091	M	—	—	—	—	C
5659	M	—	—	—	—	C
5667	M	—	—	—	—	C
6897	M	—	—	—	—	C
7564	F	—	—	—	—	C

[a] M = mangabey origin; H = human origin.
[b] L = lepromatous; I = indeterminate, R = resistant; C = control. Clinical status as of 1 January 1985.

ferentially centrifuged. Bacilli were counted as described by Shepard ([10]). Human-derived *M. leprae* were obtained from armadillo tissue inoculated with human isolates.

The clinical status of all animals was as of January 1985. Animals diagnosed as having lepromatous leprosy had dermal histiocytic infiltrates which contained numerous acid-fast bacilli (AFB), had nerve involvement, and had AFB in nasal smears.. Those animals classified as having indeterminate leprosy had sparse lymphohistiocytic dermal infiltrates with rare AFB in the infiltrate or cutaneous nerves and negative nasal smears. Resistant animals had no histologic evidence of active leprosy, no AFB in the tissues, and negative nasal smears. Control animals had never been inoculated with *M. leprae.*

Lepromins for the study were prepared at the Armed Forces Institute of Pathology, Washington, D.C., U.S.A., using standard methods. Briefly, a weighed sample of tissue was boiled for 30 min and, after grinding and centrifugation, was counted. After adjusting the count to the desired number of organisms, all preparations were autoclaved and 0.5% phenol was added as a preservative. The lepromins prepared and used in this study consisted of: a) three concentrations of lepromin A prepared from subcutaneous lepromas harvested from armadillos infected with human-derived *M. leprae* with the counts adjusted to 1.6×10^8 AFB/ml (1X lepromin A), 1.6×10^9 AFB/ml (10X lepromin A), and 2.4×10^9 AFB/ml (15X lepromin A); b) normal tissue mock lepromin consisting of subcutaneous tissue harvested from a normal armadillo and processed following the same procedure used for preparing lepromin A; c) lepromin prepared in the same manner as lepromin A, but using cutaneous lepromas harvested from mangabey monkeys with lepromatous leprosy (designated lepromin M); and d) purified preparations of inactivated armadillo-passaged human *M. leprae* free of tissue contaminants (provided by Dr. Patrick Brennan, Colorado State University) and designated as 1X (1.6×10^8 AFB/ml) and 25X (4.0×10^9 AFB/ml) purified lepromin.

Lepromin tests were performed by intra-

TABLE 2. *Clinical measurements of lepromin reactions (mm).*[a]

Animal no.	Disease state[b]	Skin test preparation[c]															
		3 days								25 days							
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
A125	L	2	0	1	5	0	0	0	1.5	0	0	0	0	0	0	0	2
8664	L	2	0	0	0	0	0	0	ND[d]	1	0	0	0	0	0	0	ND
B988	L	4	0	0	2	0	0	0	ND	0	0	0	0	0	0	0	ND
B465	L	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	11
B539	I	2	0	0	3	0	0	0	ND	4	4	2	6	0	0	0	ND
A749	I	2	2	0	4	0	0	0	3.5	0	0	0	4	0	0	0	5
B685	R	2	0	0	3	0	0	0	2	6	6	3	20	2	0	0	11
B960	R	5	2	1	5	3	0	0	3	10	10	3	20	3	0	0	15
B849	R	6	0	2	4	2	0	0	2.5	6	8	2	15	0	0	0	7
B630	R	3	2	1	3	0	0	0	2	4	4	1	6	0	0	0	8
B614	R	7	5	4	4	0	0	0	2.5	2	1	2	4	0	0	0	10
B784	R	7	5	3	4	2	0	0	2.5	6	6	2	10	0	0	0	8
A491	R	5	2	0	5	0	0	0	2	6	4	0	10	0	0	0	18
B748	R	1	3	1	4	3	0	0	ND	4	3	3	4	0	0	0	ND
B845	R	1	0	1	3	0	0	0	ND	3	4	1	10	0	0	0	ND
6897	C	3	1	1	5	0	0	0	ND	0	0	0	2	0	0	0	ND
9091	C	3	0	0	2	0	0	0	2.5	1	0	0	4	0	0	0	1.5
5667	C	1	0	2	5	1	0	0	ND	1	0	0	2	0	0	0	ND
9101	C	2	0	0	2	0	0	0	0	1	0	0	6	0	0	0	1.5
B244	C	5	2	2	4	0	0	1	2.5	0	4	0	5	0	0	0	2
5659	C	2	2	0	3	0	0	0	ND	0	0	0	0	0	0	0	ND

[a] Greatest diameter of induration, mm.
[b] L = lepromatous; I = indeterminate; R = resistant; C = control.
[c] 1 = 25X purified lepromin; 2 = 1X lepromin A; 3 = 1X lepromin M; 4 = 10X lepromin M; 5 = 1X purified lepromin; 6 = saline control; 7 = armadillo mock lepromin; 8 = 15X lepromin A.
[d] ND = not done.

cutaneous inoculation of 0.1 ml of the various preparations on the abdomen. In March 1983, 15X lepromin A was inoculated into 4 infected, 10 resistant, and 3 control monkeys. In March or June 1984, 1X lepromin A, 10X lepromin A, 1X lepromin M, 1X purified lepromin, 25X purified lepromin, armadillo mock lepromin, and a saline control were administered simultaneously at separate sites on the abdomen to 6 infected, 12 resistant, and 7 control monkeys, including those previously tested in March 1983. The inoculation sites were examined at 24 hr and at 3 weeks postinoculation, and any reactions measured across the greatest diameter.

The inoculation sites were entirely excised at 28 days postinoculation. The tissues were fixed in 10% neutral buffered Formalin, bisected through the greatest dimension of any reaction, processed in paraffin, cut at 6 μm, and stained with hematoxylin and eosin (H&E) and Fite-Faraco acid-fast stains. The following parameters were evaluated individually for each injection site: a) size of infiltrate, b) number of AFB, c) necrosis, and d) degree of epithelioid change in histiocytes. The size of the lesions was evaluated by measuring the maximum length and height of the area of infiltration on histologic slides (which were taken through the maximum diameter of the lesions) and multiplying to obtain an area. This is only an approximation since the lesions were not rectilinear. Shrinkage of tissue during processing was considered constant. Areas of inflammatory cell infiltration smaller than 1.0 mm^2 were not measured and were recorded as 0. The numbers of residual AFB at the skin-test sites were subjectively evaluated on Fite-Faraco-stained slides, utilizing a logarithmic scale from 1+ (1 to 10 AFB per slide) to 6+ (>1000 AFB per oil immersion field). Bacilli were not uniformly distributed throughout the lesions. Therefore, the area of highest concentration of AFB was evaluated, even if it encompassed only a few high-power fields. Necrosis was evaluated on a scale of 0 (none present) to 4+, with 1+ being an area of necrosis approximately one high-power field or less in diameter and 4+ being an area of

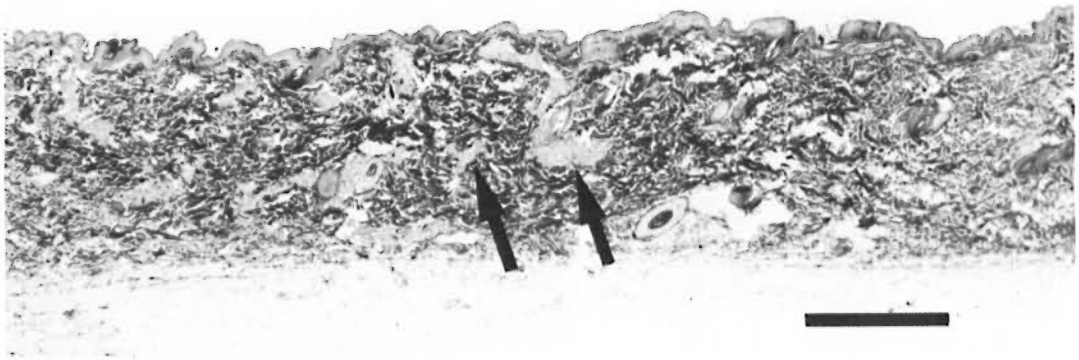

FIG. 1. Negative lepromin test (using 10X lepromin A) in a rhesus monkey with experimentally induced lepromatous leprosy. Note sparse dermal histiocytic infiltrate (arrows). This reaction is <1 mm^2 and was read as 0 (H&E $\times 12.5$). Bar = 1 mm.

necrosis >2.0 mm in diameter. Epithelioid change was subjectively evaluated on a scale of 0 to 4+, with 1+ being a loose accumulation of histiocytes with small nuclei and scant cytoplasm with well defined borders and 4+ being a well-developed tuber-

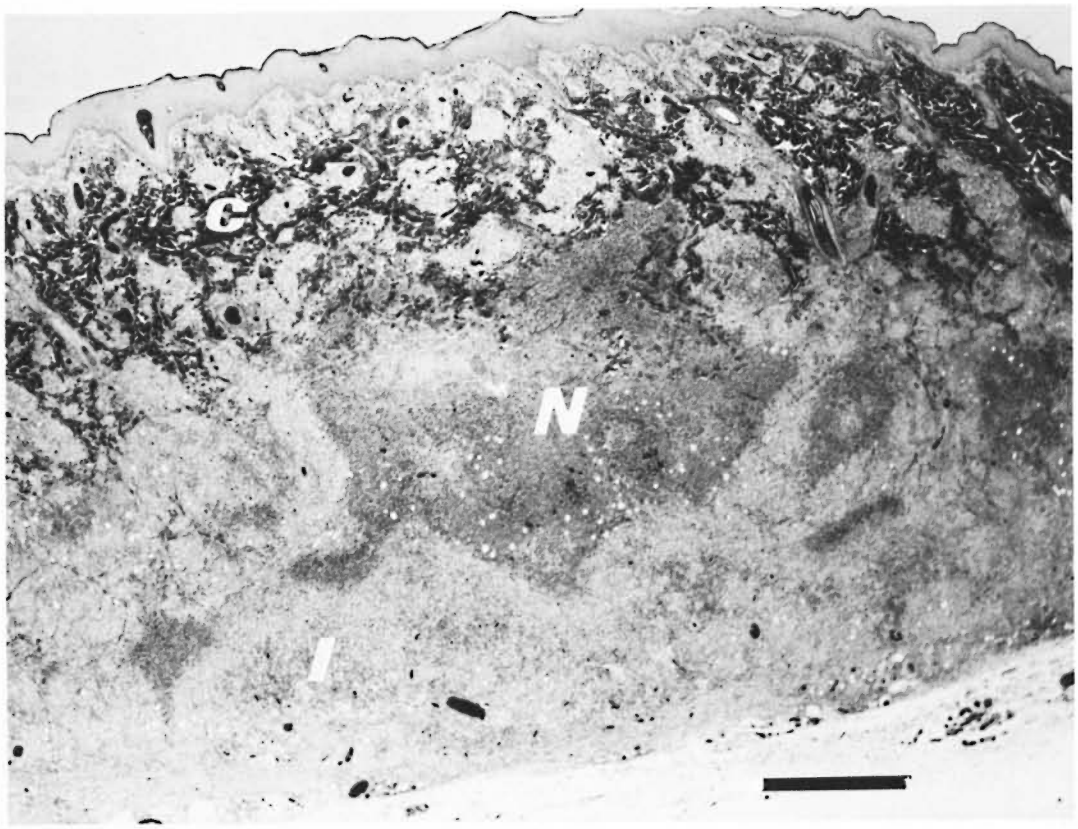

FIG. 2. Strongly positive lepromin test (using 10X lepromin A) in a rhesus monkey resistant to experimentally induced leprosy. Note extensive dermal infiltrate (I) and central area of necrosis (N); dark areas are dermal collagen (C) (H&E $\times 12.5$) Bar = 1 mm.

culoid granuloma in which histiocytes had large nuclei with prominent nucleoli, abundant cytoplasm with indistinct margins, and in which multinucleate giant cells were present.

Statistical evaluation was performed using a two sample *t* test to evaluate the mean scores of infected (lepromatous and indeterminate combined), resistant, and control animals for the various histological parameters.

RESULTS

The results of the clinical measurements of the lepromin reactions are shown in Table 2. At 3 days, the inoculation sites were erythematous and slightly thickened, and by 25 days, reactive sites were erythematous, indurated, and often raised. The large reactions often had ulcerated centers.

Histologically, the reactions varied qualitatively and quantitatively. Some injection sites showed only a few small lymphocytic cuffs around dermal capillaries. Slightly more extensive reactions combined perivascular cuffing with sparse infiltrates of histiocytes and lymphocytes within the dermis (Figs. 1 and 3), but these responses were small and poorly organized. Larger reactions consisted of perivascular cuffing of dermal vessels and discrete infiltrates consisting of intermixed histiocytes and lymphocytes. The size of the reactions was directly proportional to the degree of epithelioid change in the macrophages, the number of multinucleated giant cells, and the amount of necrosis within the infiltrate (Figs. 2 and 4). Many reactions to 15X lepromin A were ulcerated. When present, AFB were in histiocytes or in areas of necrosis, and in lesions which contained areas of necrosis, AFB were often in highest concentration in the necrotic areas. None of the animals reacted to armadillo mock lepromin or to the saline control.

The mean scores for the various histological parameters evaluated for each lepromin preparation are shown in Table 3. Histograms of lesion size distribution are shown in Figure 5.

With 15X lepromin A, administered in March 1983, positive reactions were large and many were ulcerated. Two of the four infected animals reacted to the lepromin. Therefore, only the difference in size be-

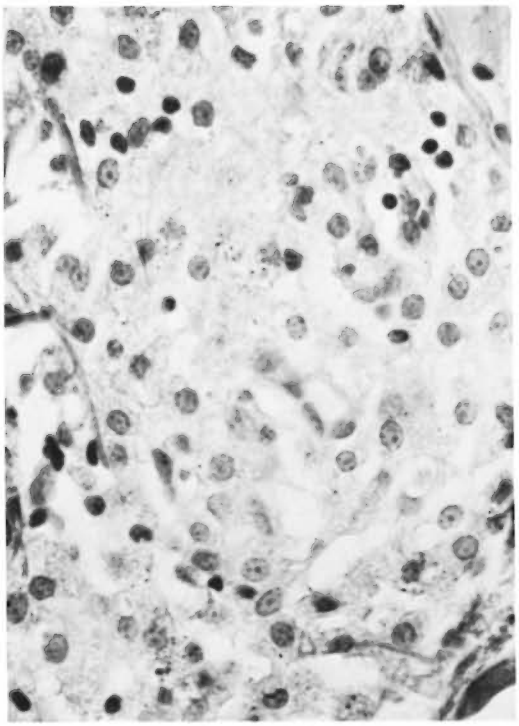

FIG. 3. Higher magnification of Figure 1. Note histiocytic infiltrate (H&E ×250).

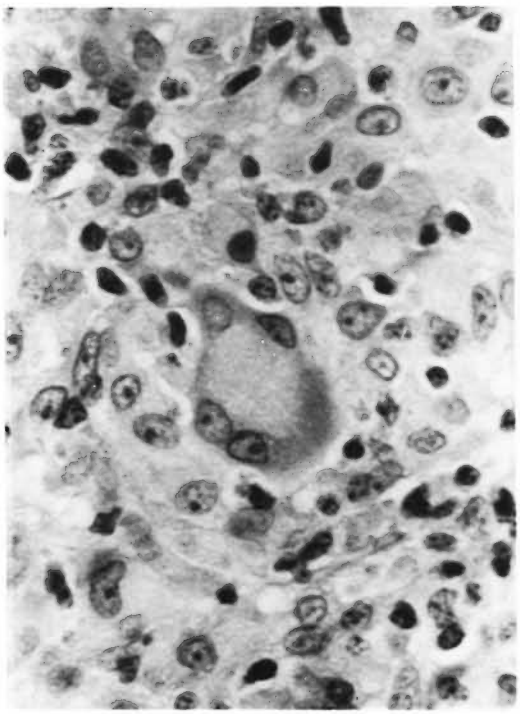

FIG. 4. Higher magnification of Figure 2. Note epithelioid cell infiltrate and giant cell (H&E ×250).

tween resistant and control animals was statistically significant ($p < 0.0001$), while other size differences were not. The differences in the other parameters were not statistically significant. The histogram (Fig. 5) failed to clearly separate infected from resistant animals.

Inoculation of 1X lepromin A, administered approximately one year later, resulted in small reactions, and the three groups of animals were not clearly separated on the histogram. The difference in the sizes of reactions was significant between infected and resistant animals ($p = 0.015$). There was also a significant difference between the numbers of AFB in the lesions in infected and control monkeys ($p = 0.024$), but not between other groups. There was more epithelioid change ($p < 0.0001$) in the resistant than in the infected animals.

The results with 10X lepromin A showed the clearest relationship between clinical status and character of reaction. The histogram (Fig. 5) shows that the sizes of the reactions separate the infected and control animals from those inoculated animals which were resistant and did not develop the disease. Differences in the sizes of lesions between resistant and control ($p = 0.0012$) monkeys and resistant and infected ($p = 0.011$) monkeys were statistically significant. There were significantly more AFB in control animals than in resistant animals ($p < 0.0001$). There was also more necrosis in resistant animals, and this change was correlated with reaction size. Resistant animals had significantly more epithelioid changes than did infected animals, but the difference between resistant and control monkeys was not significant.

With 1X lepromin M, the histogram (Fig. 5) separates resistant monkeys from infected and control monkeys fairly well. The resistant animals had significantly larger reactions ($p = 0.0047$). However, such reactions were probably too small to be useful in practice. The control animals had significantly more AFB in their lesions than did other groups ($p = 0.022$). Resistant animals had more epithelioid change than did infected monkeys ($p = 0.0019$).

The reactions with 1X purified lepromin were quite small. The histogram (Fig. 5) does not separate the groups well, and the differences in histological parameters are too small to be of practical use.

The 25X purified lepromin separated the animals slightly better, although many resistant animals had small reactions and the histogram (Fig. 5) does not clearly separate the groups. The mean size of the lesions in resistant animals was significantly larger than in the control or infected groups, although there was some overlap between groups at the lower end of the scale (Fig. 5). The control and infected monkeys had significantly more AFB in the reactions than did resistant animals. Resistant monkeys had significantly more epithelioid change than infected, but not control animals.

DISCUSSION

From this study, it is apparent that lepromin preparations intended for human use contain an insufficient amount of antigen to elicit predictable reactions in rhesus monkeys. This is not unexpected, because a higher concentration of tuberculin must be used in monkeys to elicit a positive tuberculin reaction. Highly purified lepromin, even at a 25X concentration, did not cause very large reactions in many resistant animals. The antigens in less-purified lepromin preparations are apparently important in amplifying the reaction, without changing its significance. Of those evaluated, the best lepromin preparation for use in rhesus monkeys appears to be 10X lepromin A, since this preparation gave the best separation of resistant from infected and control animals based on the size of the lesions. Our results with this preparation suggest that a positive reaction would be about 10 mm^2 or greater (measured histologically). The correlation between clinical and histological measurements of reactions was not complete. With 10X lepromin A, reactions <3 mm in diameter were negative histologically, while reactions >10 mm in diameter were positive. There was some overlap in reactions measuring from 4 mm to 6 mm in diameter clinically, with some being positive histologically and some negative. The discrepancy could be due to errors in measuring the maximum extent of reaction, either his-

TABLE 3. *Histological grading of lepromin reactions.*[a]

	Size (mm^2)	AFB (+'s)	Necrosis (+'s)	Epithelioid change (+'s)
15X Lepromin A				
Control (3)[b]	1.67 ± 1.2[c]	5.33 ± 0.3	0.0	0.0
Infected[d] (4)	8.00 ± 5.7	4.75 ± 0.2	1.00 ± 2.0	0.50 ± 0.5
Resistant (10)	19.30 ± 3.4	4.20 ± 0.3	2.20 ± 1.3	1.50 ± 0.22
1X Lepromin A				
Control (7)	0.0	2.29 ± 0.57	0.0	0.71 ± 0.36
Infected (6)	0.50 ± 0.5	0.50 ± 0.34	0.0	0.17 ± 0.17
Resistant (12)	8.33 ± 2.7	0.92 ± 0.38	0.67 ± 0.33	1.58 ± 0.26
10X Lepromin A				
Control (7)	1.43 ± 0.61	4.14 ± 0.26	0.14 ± 0.14	1.00 ± 0.38
Infected (6)	2.50 ± 1.50	3.33 ± 0.80	0.0	0.17 ± 0.17
Resistant (12)	26.70 ± 5.80	1.50 ± 0.31	2.08 ± 0.36	1.92 ± 0.15
1X Lepromin M				
Control (7)	0.0	1.43 ± 0.43	0.0	0.57 ± 0.30
Infected (6)	0.33 ± 0.33	0.0	0.0	0.17 ± 0.17
Resistant (12)	2.65 ± 0.61	0.08 ± 0.08	0.25 ± 0.18	1.42 ± 0.29
1X Purified lepromin				
Control (7)	0.0	0.14 ± 0.14	0.0	0.0
Infected (6)	0.0	0.0	0.0	0.0
Resistant (12)	2.42 ± 1.48	0.08 ± 0.08	0.25 ± 0.25	0.67 ± 0.28
25X Purified lepromin				
Control (7)	0.71 ± 0.57	3.86 ± 0.34	0.0	0.71 ± 0.36
Infected (6)	1.00 ± 0.52	3.17 ± 0.65	0.17 ± 0.17	0.33 ± 0.21
Resistant (12)	10.50 ± 3.30	0.83 ± 0.24	1.33 ± 0.31	1.67 ± 0.26

[a] See text for methods of grading.
[b] Number of monkeys in group.
[c] Mean ± S.E.M.
[d] Lepromatous and indeterminate combined.

tologically or clinically. It is also possible that the extent of clinically detectable induration does not always reflect the degree of histologically apparent infiltration.

None of the preparations elicited a significant reaction in the control animals. Thus, a negative lepromin test may not have the same predictive value for susceptibility to lepromatous disease in rhesus monkeys that it does in humans. It is unclear why many presumably unexposed humans develop positive lepromin tests, while presumably unexposed rhesus monkeys do not. This may reflect differences in exposure in the environment to bacteria which have crossreacting antigens. The only animals that developed significant reactions were those which had been exposed to viable *M. leprae* by previous inoculation and had not developed any disease. It is likely that the positive response of these animals is a reflection of cell-mediated immunity caused by their previous "vaccination" with *M. leprae.* Thus, skin testing should be a useful tool in vaccine trials in monkeys, as well as in separating those monkeys which have been inoculated with *M. leprae* and which are likely to develop leprosy from those which are not. The animals with lepromatous disease failed to respond to lepromin, as expected, while those with indeterminate disease responded slightly, but less than animals without the disease. Because we have not seen rhesus monkeys with tuberculoid or borderline leprosy, we do not know what their reactions would be.

We noted the numbers of AFB within the lesions in order to evaluate the monkey's ability to clear bacilli as previously described for humans ([3]). In general, control animals were the least efficient in clearing bacilli and resistant animals were the most efficient, while infected animals were intermediate between the two. This parameter

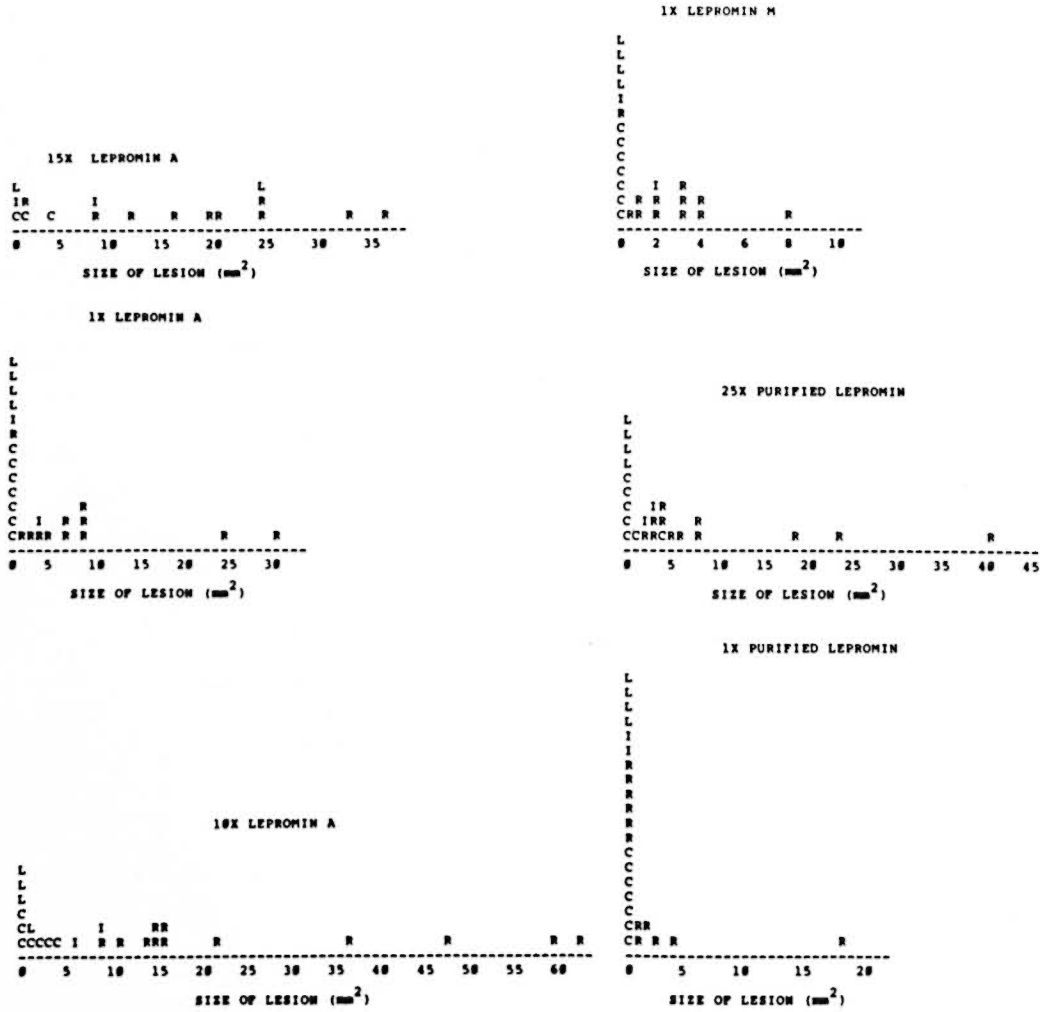

FIG. 5. Size distribution of lepromin reactions measured histologically. L = lepromatous leprosy; I = indeterminate leprosy; R = resistant to leprosy; C = uninoculated control.

seems to have little predictive value in monkeys not previously exposed to *M. leprae.*

The responses to lepromin M were similar to those with lepromin A, indicating that mangabey tissue present in the original inoculum for some of the animals is not an important factor in eliciting lepromin responses. Likewise, none of the animals responded to normal armadillo mock lepromin, suggesting that armadillo tissue antigens also played little role in sensitizing the monkeys. It has been suggested that armadillo tissue may enhance the lepromin reaction in humans ([7]).

Three of the seven control animals were tested both in 1983 (with 15X lepromin A) and in 1984 (with 1X lepromin A, 10X lepromin A, 1X lepromin M, 1X purified lepromin, and 25X purified lepromin). Their reactions after the second test were no different than the controls which had not been previously tested, indicating that previous lepromin testing had not sensitized the monkeys. A large dose of antigen was given in 1983, but the reaction was surgically removed for histology at 28 days. The effect this may have had on subsequent sensitization is unknown.

Also of interest are the two infected animals (one lepromatous and one indeterminate) which developed reactions clinically and histologically similar to those of

resistant animals when tested with 15X lepromin A in 1983. At that time neither monkey had clinically apparent disease. When tested one year later with the other lepromin preparations, the lepromatous animal did not react at all and the indeterminate animal had only a small reaction. At least in the case of the lepromatous monkey, a "positive" lepromin test was clearly not predictive of the course of infection and reversed from "positive" to "negative" as lepromatous disease developed. We have previously shown that immune function in mangabey monkeys declines concurrently with the progression of the disease ([6]).

In conclusion, the lepromin test is useful in rhesus monkeys, although its significance may be different than in humans. A higher concentration of antigen (about 10X) is necessary to elicit an easily measurable response. Monkeys which have not been experimentally exposed to *M. leprae* appear to be lepromin negative. Thus, the lepromin test cannot be used in unexposed monkeys as a test to predict susceptibility to leprosy. Monkeys which have been inoculated with *M. leprae* and which develop lepromatous disease are lepromin negative. At least one animal was initially lepromin positive after experimental inoculation with viable *M. leprae,* subsequently developed lepromatous leprosy, and was then lepromin negative. Animals which are experimentally inoculated with *M. leprae* and which do not develop the disease become lepromin positive. Thus, the lepromin test in rhesus monkeys appears to be a good indicator of acquired cell-mediated immunity to *M. leprae.*

SUMMARY

The lepromin test was studied in rhesus monkeys. Six control monkeys which had not been inoculated with *Mycobacterium leprae,* six monkeys with experimentally induced leprosy, and nine monkeys which had been inoculated with *M. leprae* but had not developed leprosy were evaluated with 1X, 10X, and 15X lepromin A, with 1X and 10X lepromin M (mangabey monkey derived), with 1X and 25X purified inactivated *M. leprae,* and with an armadillo mock lepromin. We found that the lepromin test is useful in rhesus monkeys, but that a higher concentration of antigen than is used in humans is required to induce a response in monkeys. Control monkeys appear to be lepromin negative. Animals which have been inoculated and which develop lepromatous leprosy are also negative. Monkeys which are experimentally inoculated with *M. leprae* and do not develop leprosy become lepromin positive. Monkeys with indeterminate leprosy have reactions intermediate between lepromatous and resistant animals. No monkeys reacted to armadillo tissue. Our results indicate that 10X lepromin A is a useful preparation for the lepromin testing of rhesus monkeys.

RESUMEN

Se aplicó la prueba de la lepromina en monos rhesus. En el estudio, 6 monos control no inoculados, 6 monos con lepra inducida experimentalmente y 9 monos inoculados con *Mycobacterium leprae* que todavia no habían desarrollado la enfermedad, recibieron lepromina A 1X, 10X y 15X, lepromina M (de mono mangabey) 1X y 10X, *M. leprae* purificado e inactivado 1X y 25X, y lepromina de un armadillo de origen incierto. Se encontró que la prueba de la lepromina es útil en los monos rhesus cuando se induce con dosis más altas que las usadas en lose humanos. Los monos control parecen ser lepromino-negativos, los animales inoculados con *M. leprae* que desarrollaron la enfermedad lepromatosa fueron también lepromino-negativos. Los monos inoculados con *M. leprae* que no desarrollaron la enfermedad llegaron a ser lepromino-positivos. Los monos con lepra indeterminada dieron reacciones intermedias. Ningún mono reaccionó con tejido de armadillo. Los resultados indícan que la lepromina A 10X es una preparación útil para le prueba de la lepromina en los monos rhesus.

RÉSUMÉ

L'épreuve à la lépromine a été étudiée chez des singes rhésus. Six singes témoins qui n'avaient pas été inoculés avec *Mycobacterium leprae,* de même que six singes atteints de lèpre transmise expérimentalement, et neuf singes qui avaient été inoculés avec *M. leprae* mais n'avaient pas développé de lésions de lèpre, ont été soumis à une série de lépromines, à savoir la lépromine A standard, des lépromines A concentrées 10 et 15 fois, de la lépromine M obtenue chez le singe mangabey, à concentration normale ou concentrée 10 fois, des bacilles de la lèpre purifiés et inactivés, en doses simples ou concentrées 20 fois, et une lépromine de tatou. On a observé que l'épreuve à la lépromine était utile chez les singes rhésus. Toutefois, une concentration supérieure d'antigène à celle utilisée chez l'homme est nécessaire pour entraîner une réponse chez les singes. Les singes témoins se révèlent être négatifs à la lépromine. Les animaux inoculés qui avaient développé une lèpre lépromateuse étaient également né-

gatifs. Les singes qui ne développent pas de lèpre après une inoculation expérimentale avec *M. leprae* deviennent également positifs à la lépromine. Les singes atteints de lèpre indéterminée présentent des réactions intermédiaires entre celles constatées chez les animaux lépromateux et chez ceux qui sont résistants. Aucun singe n'a réagi avec le tissu de tatou. Ces résultats montrent que la lépromine A concentrée 10 fois constitue un réactif utile pour pratiquer l'épreuve à la lépromine chez les singes rhésus.

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