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Variable Lepromin Response to *Mycobacterium leprae* in Resistant Armadillos[1]

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Experimentally induced lepromatous leprosy in armadillos was first reported by Kirchheimer and Storrs in 1971 ([4]). It was also shown that armadillos were highly susceptible to lepromatous disease and the prevalence of the experimental disease varied from 40%–65% in one laboratory ([6]) to 88%–93% in another ([3]). Recently it was demonstrated that animals which were lepromin negative were prone to develop the disseminated disease and those which showed the positive lepromin response were resistant to the disease ([2]). In this study, a group of eight armadillos which previously had been inoculated with *Mycobacterium leprae,* but did not develop disseminated or localized leprosy and therefore were considered resistant to the infection, were lepromin tested to further elucidate the relationship of cell-mediated immunity (CMI), as shown by a lepromin test, to the development of the disease.

MATERIALS AND METHODS

Eight resistant armadillos were collected from four different experiments (Table 1). In one, 20 armadillos were infected intradermally with 10^3 *M. leprae.* Of the three which did not develop disseminated disease five years after infection, one was available for study. In the second experiment, 20 armadillos were infected intradermally with 10^4 *M. leprae.* Of the six which were resistant to the disease five years after inoculation, four were available for study. In the third experiment, 10 animals were infected with 10^5 *M. leprae* intradermally and of the 3 which are resistant seven years after inoculation, 2 were available for study. In the fourth experiment, five animals were infected intradermally with 2×10^8 *M. leprae.* Of these, the one resistant animal seven years after inoculation was available for study.

All eight animals were lepromin tested at two sites in the abdominal skin by injecting armadillo-derived integral lepromin containing 1.6×10^7 heat-killed *M. leprae* intradermally. The reaction at the end of 48 hr was measured in all animals and biopsied in five. The reaction at the other site was measured and biopsied in all eight animals after four weeks. At the end of this study the animals were again injected at the abdominal skin intradermally with 1.6×10^7 live armadillo-derived *M. leprae.* The reaction to the inoculation at the site was measured at 28 days and was biopsied.

Two animals (40 and 298) which gave the largest reaction to lepromin were chosen and were inoculated intradermally with killed *M. leprae* at doses of 10^3, 10^4, 10^5, 10^6, and 10^7 at five different sites. The skin reactions were measured at the end of 28 days and were biopsied.

The test sites were identified by tatooing India ink around the injected site. All biopsies were fixed in 10% buffered formalin and processed for paraffin sections, and sections were made at 5 μ thickness. One was stained with hematoxylin and eosin (H & E) and another with a modified Fite stain for *M. leprae* and then examined.

RESULTS

Histopathology of lepromin reaction

Reaction at 48 hr. The biopsy of the test site at 48 hr was examined in five animals. One of the five had a negative reaction macroscopically. Histological examination showed a well localized reaction in the dermis of all five animals (Fig. 1). There was a central area containing numerous neutrophils, some macrophages, and a few lymphocytes (Fig. 2). Away from the necrotic

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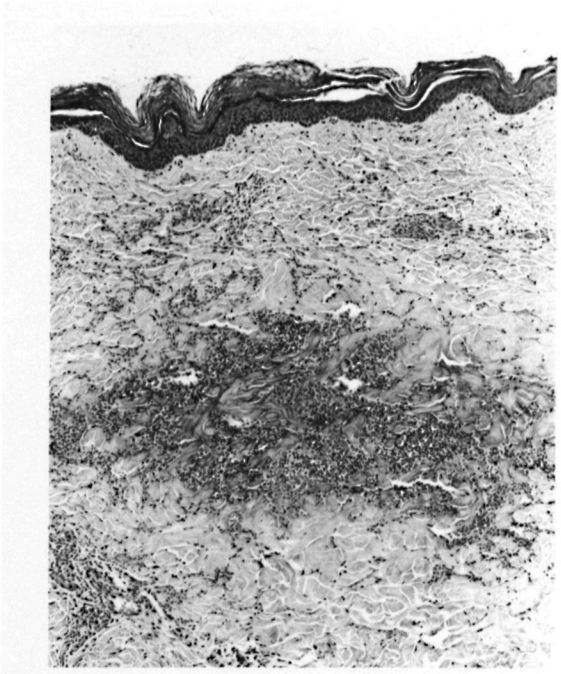

FIG. 1. A 48 hr lepromin reaction of an armadillo with a tuberculoid reaction. There is a localized area of necrosis infiltrated by numerous neutrophils, some macrophages, and lymphocytes. (H & E ×75).

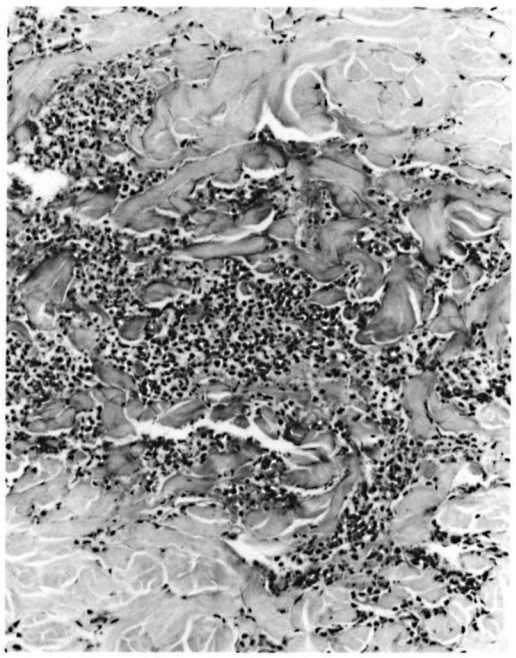

FIG. 2. High power of Figure 1. Note the numerous neutrophils infiltrating the collagen bundles. (H & E ×200).

area there were a few newly formed capillaries with perivascular lymphocytes. The size of the reaction varied from 1 mm–3 mm. Acid-fast stain showed clumps of organisms in the necrotic area lying free in tissue and also inside neutrophils and macrophages. The three animals which at 28 days showed tuberculoid reactions had much bigger reactions than the two which exhibited borderline reactions.

Reaction at 28 days. The reaction can be classified into three patterns: tuberculoid, borderline, and lepromatous.

Tuberculoid pattern. Five of the eight animals were in this group and, macroscopically, all five showed a positive reaction. Histologically it was well circumscribed and was confined to the dermis. There were one or more large areas of necrosis of collagen (Fig. 3). The necrosis resembled more the fibrinoid type than the caseous and was present in four of the five animals in this group. The necrotic area was surrounded by large clumps of epithelioid cells (Fig. 4). Scattered among them were lymphocytes and occasional Langhan's giant cells. Acid-fast stain showed some bacilli in the necrotic area lying freely in the tissue. Some of the macrophages immediately surrounding the necrotic area also contained a few bacilli in all except one animal.

TABLE 1. *Resistant armadillos studied.*

Expt. no.	No. armadillos	Infective dose of *M. leprae*	No. years after inoculation	No. resistant	No. available for study
1	20	10^3	5	3	1
2	20	10^4	5	6	4
3	10	10^5	7	3	2
4	5	2×10^8	7	1	1
Total					8

Borderline pattern. Two of the animals belonged to this group, and both had negative macroscopic reactions. Histologically there was no necrosis. Well-circumscribed collections of macrophages and immature and mature epithelioid cells, occasional Langhan's giant cells, and numerous lymphocytes were seen (Figs. 5 & 6). (Immature epithelioid cells are those which morphologically resemble epithelioid cells but on acid-fast stain continue to show fragments

FIG. 3. A 28 day lepromin reaction of an armadillo with a tuberculoid reaction. Note the large area of necrosis of collagen walled off by a granuloma. (H & E ×40).

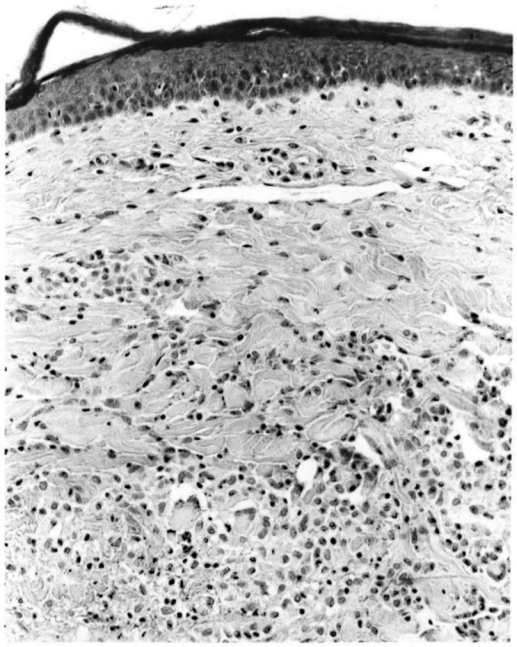

FIG. 4. High power of Figure 3. The granuloma surrounding the necrotic area consists of numerous epithelioid cells, Langhan's giant cells, and some lymphocytes. (H & E ×300).

of acid-fast bacilli in their cytoplasm.) Acid-fast stain showed bacilli in most of the macrophages and immature epithelioid cells. In some there were many bacilli and in others, there were only a few.

Lepromatous pattern. The one animal that belonged to this group showed macroscopically a negative reaction. Histologically in the dermis there were a few small collections of mononuclear cells consisting mainly of macrophages and a few lymphocytes (Figs. 7 & 8). Acid-fast stain showed macrophages loaded with acid-fast organisms. A few scattered macrophages which were present around capillaries many fields away from the area of reaction also contained many bacilli. There was no attempt to localize the reaction. The macrophages did not show any foamy change.

Histopathology of the reaction to live *M. leprae*

The tissue response to 1.6×10^7 live *M. leprae* at the end of 28 days was studied in all eight animals. Macroscopic readings showed very little variation from that of killed *M. leprae.* The histopathological reaction was almost identical and could not be differentiated from that of killed *M. leprae.* In the animal which belonged to the third group, showing a lepromatous response, there was no macroscopic reaction. The histopathology of the biopsy from the site of inoculation did not show any localized cellular reaction. Only a few scattered macrophages containing bacilli were seen in the dermis.

Histopathology of the reaction to different doses of lepromin

Dose of 10^3. The reaction was macroscopically negative, and the tissue response was confined to a very small area in the dermis. Several small clumps of epithelioid cells and a few scattered lymphocytes were present. Acid-fast stain showed no bacilli.

Doses of 10^4 and 10^5. The tissue response was confined to the dermis and the cellular components were similar to that of the smaller dose of 10^3. There was an increase in size of the granuloma which was slightly more in the 10^4 dosage and much more in the 10^5 dosage. No organisms were seen on acid-fast stain in either dosage.

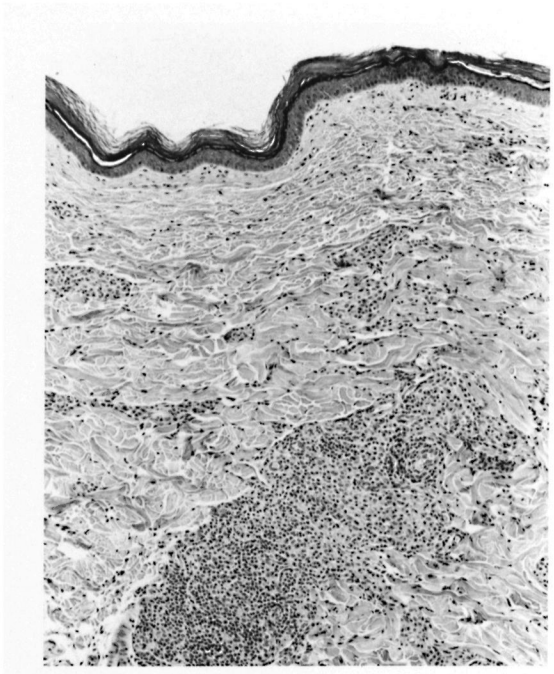

FIG. 5. A 28 day lepromin reaction of an armadillo with a borderline reaction. Note the granulomatous reaction in the dermis in focal areas. (H & E ×75).

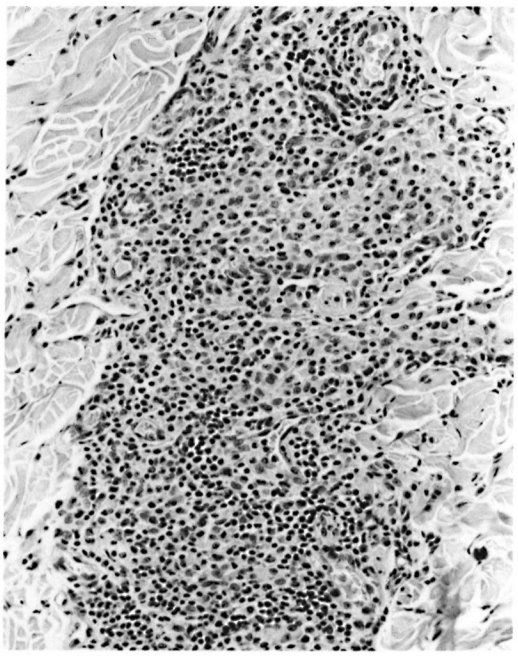

FIG. 6. High power of Figure 5. The granuloma consists of immature epithelioid cells and many lymphocytes. (H & E ×300).

Doses of 10^6 and 10^7. There were well-circumscribed granulomas in the dermis with a central area of fibrinoid necrosis of the collagen surrounded by numerous epithelioid cells and diffusely scattered lymphocytes. Acid-fast stain showed bacilli lying free in the necrotic area and inside immature epithelioid cells immediately surrounding the necrotic area. The reaction in the 10^7 dosage was larger than that of the 10^6, but the cellular components were similar.

DISCUSSION

Of the 8 armadillos which were resistant to infection by *M. leprae,* 5 showed tuberculoid, 2 borderline, and 1 a lepromatous reaction to *M. leprae.* This confirmed an earlier study ([2]) indicating that the armadillo is capable of developing a positive re-

TABLE 2. *Lepromin reactions—macroscopic and microscopic readings.*

Armadillo no.	Infective dose of *M. leprae*	48 hr lepromin (mm)	28 day lepromin (mm)	28 day reaction to live *M. leprae*	Histopath. evaluation
40	2×10^8	Neg. (not biopsied)	5 × 5	5 × 5	Tuberculoid
225	10^5	3 × 3	8 × 10	3 × 3	Tuberculoid
229	10^5	8 × 8	12 × 10	8 × 8	Tuberculoid
288	10^4	Neg.	Neg.	Neg.	Borderline
298	10^4	12 × 13 (not biopsied)	11 × 13	8 × 8	Tuberculoid
301	10^4	8 × 7	13 × 10	10 × 12	Tuberculoid
302	10^4	Neg. (not biopsied)	Neg.	Neg.	Lepromatous
258	10^3	3 × 3	Neg.	Neg.	Borderline

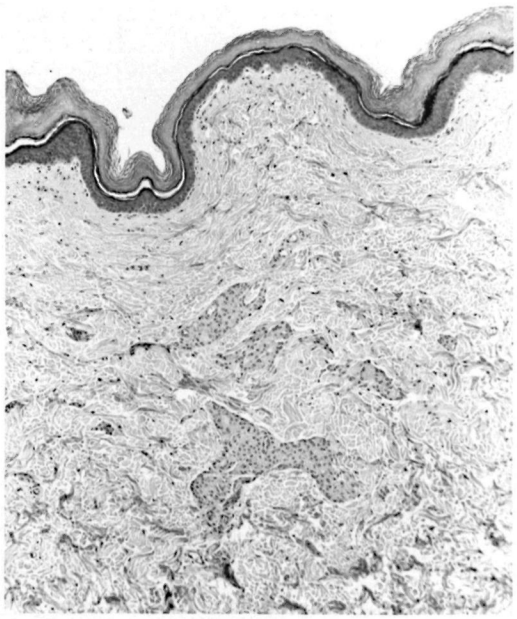

FIG. 7. A 28 day lepromin reaction of an armadillo with a lepromatous reaction. There are small collections of mononuclear cells in the dermis. (H & E ×75).

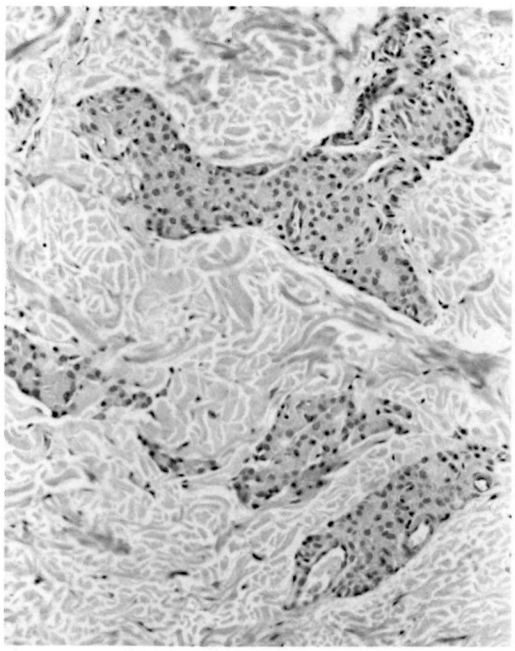

FIG. 8. High power of Figure 7. The collections of cells consist almost entirely of macrophages. No foamy change is seen. (H & E ×300).

sponse to lepromin, although the number of such animals is very small. The histopathology of the positive lepromin test showed an epithelioid cell granuloma very similar to that seen in humans. However, instead of caseous necrosis, a central area of fibrinoid necrosis of collagen was seen. Some of the injected bacteria were sequestrated in this area of necrosis surrounded by the granuloma which seemed to form a wall around it, localizing the antigen to the injected site and preventing it from being removed while they were being digested by the epithelioid cells.

Two of the resistant animals had a borderline response and one had a lepromatous response. This finding is difficult to reconcile with the resistance these armadillos had exhibited to infection by *M. leprae.* There is no doubt that the presence of CMI, as evidenced by a good positive lepromin response in five animals, is associated with protective immunity to *M. leprae.* How is it that the three animals, even with an inadequate lepromin response with immature or no epithelioid cell formation, were resistant to the infection even after several years? If lepromin response is the only expression of protective immunity to *M. leprae,* all resistant animals should have had a tuberculoid response to the lepromin test. Therefore, it is reasonable to suggest that there may be other factors not yet understood which are responsible for protective immunity to *M. leprae* in the armadillo. It should perhaps be noted that in animals with an innate immunity to infection, there need not be a cell-mediated immune re-

TABLE 3. *Lepromin readings according to the dose of* M. leprae.

Armadillo no.	Reaction to 10^3 (mm)	Reaction to 10^4 (mm)	Reaction to 10^5 (mm)	Reaction to 10^6 (mm)	Reaction to 10^7 (mm)
40	Neg.	Neg.	3 × 4	7 × 7	10 × 10
298	Neg.	4 × 4	7 × 8	11 × 12	15 × 17

sponse. In these animals, the foreign antigens can be removed from the infected site by neutrophils and macrophages and destroyed by the reticuloendothelial system.

Another interesting observation is the presence of some of the injected bacteria lying in the necrotic tissue surrounded by a large number of epithelioid cells at the inoculated site in tuberculoid reaction; whereas in borderline or lepromatous reactions, there were small clumps of macrophages packed with bacilli. From the appearance of the 48 hr reaction, it seems that there was active migration of neutrophils and macrophages to the infected site in all animals showing tuberculoid and borderline reactions. The following hypothesis is suggested to explain the subsequent events in a lepromin reaction: Macrophages that migrated and continued to migrate into the site in the tuberculoid reaction processed the antigens to present them to lymphocytes and were immobilized at the site. They remained to digest the antigens and to prevent those which were not digested from being moved out. The macrophages that migrated to the site in the borderline reaction ingested the organisms in large numbers. They could not adequately process them for presentation to lymphocytes, but carried them away as they moved out of the site of inoculation. Since there were no more free antigens there was no further migration of macrophages to the site. The few macrophages that had remained showed large clumps of organisms in their cytoplasms.

In lepromatous patients, Ward, *et al.* ([7]) have shown defective leukotaxis, and Azulay has reported deficient chemotaxis of monocytes ([1]). On the basis of our studies, it seems reasonable to suggest that the negative lepromin reaction may be due not to the inhibition of chemotaxis of neutrophils or macrophages but to the inadequate processing of antigens by macrophages, perhaps due to the absence of sensitized T lymphocytes. The response to live *M. leprae* is similar to the reaction to lepromin which contains killed *M. leprae*. This may be due to the fact that even in an inoculum of so-called live bacilli usually less than 10% of them are thought to actually be viable. It also confirms earlier findings ([5]) that autoclaving does not reduce the antigenicity of *M. leprae*.

It is also shown that the cellular content of lepromin reaction is the same if the numbers of bacilli are above 1000, but the size of the reaction increases as the dosage increases.

SUMMARY

Eight armadillos resistant to the infection of *Mycobacterium leprae* were lepromin tested. The tissue response was tuberculoid in 5, borderline in 2, and lepromatous in 1, thus showing a wide variation. It is seen that although cell-mediated immunity as evidenced by a tuberculoid granulomatous response to killed *M. leprae* is associated with resistance to the disease, there may be other yet unknown factors which protect armadillos from the infection.

Lepromin responses were recognized histologically even at a low dose of 10^3 organisms, and the response increased with the dose up to 10^7 organisms. The tissue reaction to live organisms was the same as that to killed ones, and autoclaving of *M. leprae* produced no change in the tissue response to the antigens of *M. leprae*.

RESUMEN

Se hizo la prueba de la lepromina en 8 armadillos resistentes a la infección por el *Mycobacterium leprae*. La respuesta tisular fue del tipo tuberculoide en 5 casos, intermedia en 2, y lepromatosa en 1, mostrando así una gran variación. Se ve que aunque la inmunidad celular esta associado con resistencia a la enfermedad (según se deduce por la respuesta granulomatosa tuberculoide hacia los antígenos del *M. leprae* muerto), deben existir otros mecanismos, por ahora desconocidos, que protegen a los armadillos de la infección.

Las respuestas a la lepromina fueron reconocidas histológicamente aún a dosis bajas de organismos (10^3) y aumentaron proporcionalmente con la dosis hasta 10^7 microorganismos. La reacción tisular hacia organismos vivos fue igual que hacia los organismos muertos y el tratamiento al autoclave del *M. leprae* no produjo cambios en la respuesta tisular a los antígenos del microorganismo.

RÉSUMÉ

On a procédé à l'épreuve à la lépromine chez huit tatous résistant à l'infection par *Mycobacterium leprae*. La réponse tissulaire était tuberculoïde chez 5 de ces animaux, dimorphe chez 2, et lépromateuse chez le dernier, ce qui témoigne d'une large variation dans les réactions. On constate dès lors qu'en dépit du fait qu'une immunité à médiation cellulaire, mis en évidence par une réponse granulomateuse tuberculoïde à l'inoculation de bacilles de la lèpre tués, est associée avec une

résistance à la maladie, il peut également exister d'autres facteurs inconnus qui protègent les tatous de l'infection.

Les réponses à la lépromine ont pu être mises en évidence histologiquement même à des doses faibles de 10^3 organismes. La réponse augmentait avec des doses allant jusqu'à 10^7 organismes. La réaction tissulaire aux organismes vivants était la même que celle observée à la suite d'inoculation de bacilles tués. Le passage de *M. leprae* à l'autoclave n'a pas entraîné de modifications dans les réponses tissulaires aux antigènes de *M. leprae.*

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