INTERNATIONAL JOURNAL OF LEPROSY

Volume 50, Number 2
Printed in the U.S.A.

Tissue Response to Lepromin, an Index of Susceptibility of the Armadillo to *M. leprae* Infection—a Preliminary Report[1]

Charles K. Job, Waldemar F. Kirchheimer, and Rita M. Sanchez[2]

The late lepromin reaction, named after the Japanese leprologist Kensuke Mitsuda ([8]), when positive, consists of a nodular infiltration at the site of the injection of killed *Mycobacterium leprae*. Histologically, there is a characteristic tuberculoid granuloma composed of collections of epithelioid cells, Langhans' giant cells and lymphocytes ([11]). Such a reaction indicates the presence of delayed hypersensitivity to the antigen ([14]) and means that the patient can mount a cell-mediated immune response to *M. leprae* ([16]). In a negative reaction instead of an epithelioid cell response, there is a histiocytic or a fibroblastic reaction ([1, 10, 12, 13]). In a more recent study it has been shown that the histologically evaluated lepromin reaction gives a fairly good assessment of the immunological status of the patient and that there is great similarity between the histological reaction of the lepromin test and the histopathological appearance of the skin lesion ([15]). Dharmendra and Chatterjee, in testing the prognostic value of lepromin, arrived at the conclusion that "those with a negative reaction are more likely both to develop the disease and to get it in the more serious form."([2])

In this preliminary report, a retrospective study of the histopathological appearance of the lepromin reaction of the armadillo in relation to its response to an infecting dose of *M. leprae* is recorded.

MATERIALS AND METHODS

In one experiment, 14 armadillos were skin tested using lepromin prepared from human material. *M. leprae* (3.8×10^7) suspended in 0.2 ml of saline were injected intradermally and the site was identified by tattooing India ink around the spot. The reaction site was biopsied with a 6 mm sterile biopsy punch after 28 days. The specimens were fixed in 10% formalin; processed for paraffin sections; cut at 5 μ thickness; stained with acid-fast stain for *M. leprae* and hematoxylin and eosin stain, and studied. The inoculation of 1×10^7 viable *M. leprae* was carried out intracutaneously in 3 animals 10 to 18 days after the lepromin test; in 4, on the day of the test and in 7, one to five months before the test. The animals were followed up for the development of disseminated leprosy for a period varying from 13 months to 104 months. Six of the animals were found dead and eight were sacrificed.

RESULTS

The lepromin reaction

Three different types of tissue response to heat-killed *M. leprae* were recorded:

Lepromatous lepromin reaction. The skin showed no changes in the epidermis. The inflammatory reaction was confined mostly to the dermis and its extension to the subcutaneous tissue was rare. The cellular reaction was minimal and occupied less than 20% of the area of the sections. It was composed almost entirely of small collections of macrophages with round to oval nuclei and abundant cytoplasm with marked vacuolation giving rise to a foamy appearance (Fig. 1). These collections of inflammatory cells were scattered in the dermis and seemed to extend along the tissue spaces between collagen bundles. Very occasional lymphocytes were present. Acid-fast stain showed that the foamy macrophages were packed with granular acid-fast organisms (Fig. 2).

This histopathological reaction is similar

[1] Received for publication on 18 August 1981; accepted for publication in revised form on 8 January 1982.

[2] C. K. Job, B.Sc., M.D., F.R.C.Path., F.A.M.S., Chief, Pathology Research Department; W. F. Kirchheimer, M.D., Ph.D., Chief, Laboratory Research Branch; R. M. Sanchez, B.S., Microbiologist, National Hansen's Disease Center, Carville, Louisiana, U.S.A.

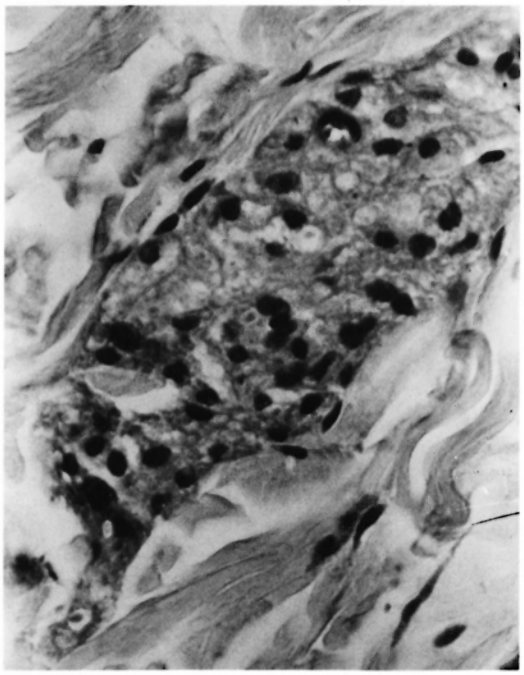

FIG. 1. A small collection of foamy macrophages in the dermis in a lepromatous lepromin reaction (H & E ×750).

to that seen in lepromatous leprosy patients and can be considered as a lepromatous lepromin reaction. Eleven of the 14 armadillos showed this type of reaction.

Borderline lepromin reaction. The skin biopsy showed no lesions in the epidermis. In the dermis there were large collections of inflammatory cells occupying approximately 50% of the area of the section. The cells were mostly histiocytes with oval to round nuclei. The cytoplasm of many of the cells was vacuolated (Fig. 3). However, in some areas there was a significant number of lymphocytes (Fig. 4). The histiocytes at these sites had a uniformly pink-staining cytoplasm and much less vacuolation. Acid-fast stain showed large clumps of bacilli in the vacuolated cytoplasm of the histiocytes (Fig. 5). The areas with lymphocytes showed fewer bacilli (Fig. 6). This histopathological appearance was consistent with that of a borderline lepromin reaction. Only one armadillo had this type of reaction.

Tuberculoid lepromin reaction. The skin biopsy showed a normal epidermis. The dermis showed large collections of epithelioid cells with oval to round vesicular nuclei and abundant, uniformly pink-staining cytoplasm. Diffusely scattered lymphocytes and several Langhans' type of giant cells were seen (Fig. 7). Over 50% of the area of the section was occupied by the granuloma. Only an occasional intracellular bacillus was seen after prolonged search in the section stained for acid-fast organisms. This histopathological picture is that of a tuberculoid lepromin reaction. Two armadillos showed this type of reaction.

Results of challenge with viable *M. leprae*.

Of the 11 armadillos with lepromatous lepromin reactions, 10 developed disseminated leprosy within a period varying from 13 to 66 months. The one which did not develop leprosy was alive at 102 months after inoculation. The only animal which had a borderline lepromin reaction was lepromin tested on the day of inoculation and it developed disseminated leprosy by 55 months after inoculation.

Of the 11 susceptible armadillos, 4 had the lepromin test done on the date of inoculation, 1 was tested 18 days before inoculation, and the other 6 at varying pe-

THE TABLE. *Relationships among the time of lepromin testing, the type of response and the susceptibility or resistance of the armadillos to the disease.*

Time of lepromin testing[a]	Lepromin reaction			No. of armadillos		Total
	Tuberculoid	Borderline	Lepromatous	Resistant	Susceptible	
10 to 18 days before	1	0	2	2	1	3
At the time of inoculation	0	1	3	0	4	4
1 to 5 months after	1	0	6	1	6	7
Totals	2	1	11	3	11	14

[a] In relation to the inoculation of viable *M. leprae*.

FIG. 2. The macrophages in a lepromatous lepromin reaction are seen packed with acid-fast bacilli (acid-fast stain ×1250).

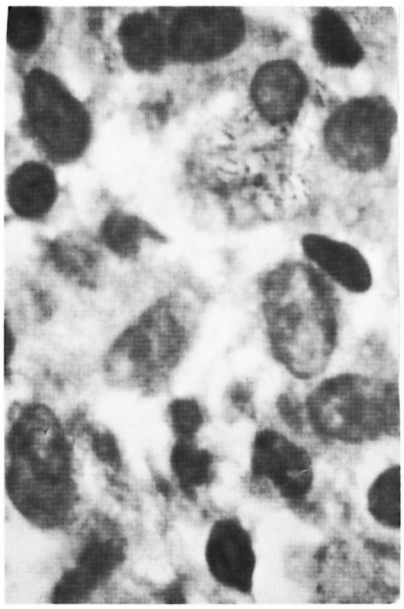

FIG. 6. The macrophages in a borderline lepromin reaction in the area of lymphocytic infiltration. Only a few of the cells contain acid-fast bacilli (acid-fast stain ×1250).

riods from one month to five months after inoculation with the infecting dose of viable *M. leprae*.

Both armadillos which had the tuberculoid lepromin reaction had not developed disease after having been followed for 104 months. One was lepromin tested ten days before and the other three months after the infective dose of *M. leprae* was given. The only armadillo with a lepromatous lepromin reaction which did not develop disseminated leprosy was lepromin tested ten days before the infective dose was given and was followed up for 102 months. Autopsies of these three resistant animals did not show any lesion suggestive of leprosy.

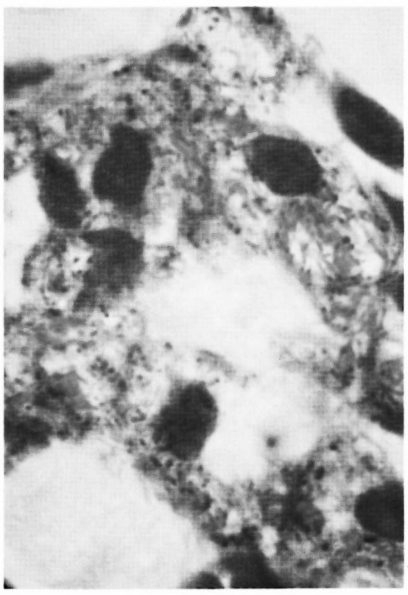

FIG. 5. The macrophages in a borderline lepromin reaction showing a large number of acid-fast bacilli filling their cytoplasm (acid-fast stain ×1250).

DISCUSSION

Kirchheimer and Storrs ([3,4]) first demonstrated that nine-banded armadillos are highly susceptible to *M. leprae*. In a recent paper, Kirchheimer reported that only 2 of 14 armadillos have not developed leprosy four years after having received an intravenous dose of 2×10^8 *M. leprae* ([6]). Although a small percentage of animals are resistant, the large majority of them are highly susceptible. However, it would be useful if the susceptible ones can be identified and differentiated from the resistant animals.

It is interesting to find in this study that

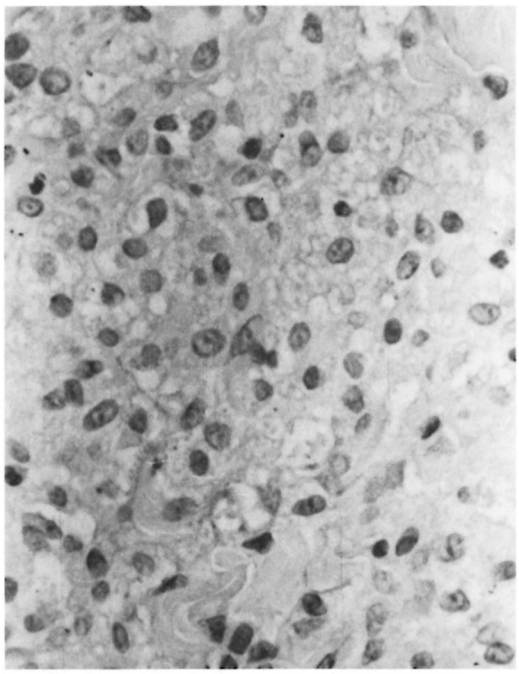

FIG. 3. A large number of macrophages in the dermis with vacuolated cytoplasm in a borderline lepromin reaction (H & E ×750).

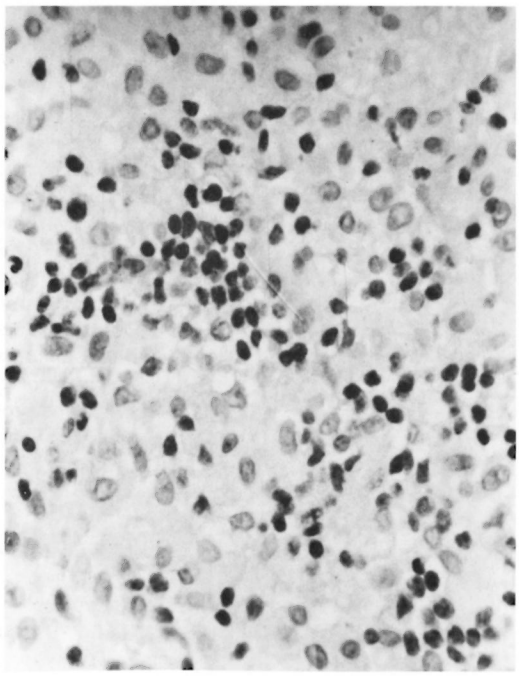

FIG. 4. A collection of macrophages in the dermis infiltrated with numerous lymphocytes in a borderline lepromin reaction (H & E ×750).

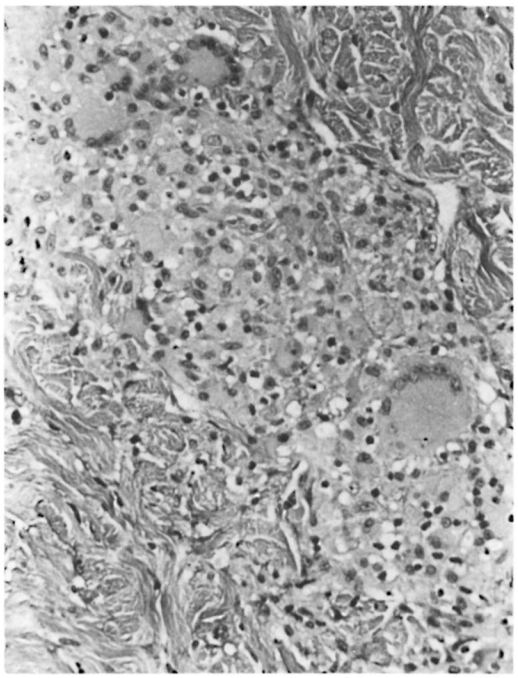

FIG. 7. A collection of epithelioid cells mixed with lymphocytes and several Langhans' type of giant cells are seen in a tuberculoid lepromin reaction. (H & E ×300).

the tissue response to killed *M. leprae* follows the same pattern in its histopathological appearance in armadillos as that of the human. A large tuberculoid granuloma with an abundant accumulation of epithelioid cells, Langhans' type of giant cells at the site of inoculation, and with almost complete clearance of *M. leprae* is characteristic of the reaction in the resistant or tuberculoid part of the spectrum of the disease. Small foam-cell foci in the mid- and deeper dermis with the cells packed with granular and beaded acid-fast organisms, which persist even after four weeks, are typical of lepromatous leprosy.

In the present study, 10 of the 11 armadillos which developed disseminated leprosy within 1 to 5½ years after inoculation with *M. leprae* showed a lepromatous lepromin reaction. Two out of the three armadillos that were resistant had a tuberculoid lepromin reaction. Although the numbers are small, the data suggest that by using the lepromin test it should be possible to differentiate the susceptible from the re-

sistant armadillos with a fair degree of accuracy. Further studies using a larger number of animals should be undertaken to confirm this impression.

Another very important fact emerges from this study. Only about 5.8% of those human contacts intimately associated with infective patients develop the disease ([9]). In comparison, 86% of armadillos infected subcutaneously with 10^4 *M. leprae* develop lepromatous leprosy. Armadillos, no doubt, are highly susceptible to leprosy. However, 14% of them develop a tuberculoid reaction to lepromin and are highly resistant to the disease. There have been many attempts to develop an effective vaccine for leprosy and now some potential vaccines are available. Those contacts of leprosy patients who need this protection are those who are most vulnerable, and are the potentially lepromatous leprosy patients.[1] Armadillos may be useful as experimental models for studying the efficacy of these vaccines. If these preparations can convert a lepromin negative armadillo to a lepromin positive one, the first step in demonstrating the efficacy of the vaccine will have been made. If the animals thus converted can be challenged with an adequate dose of *M. leprae* and found to resist the infection, the vaccine will have succeeded as a potential candidate for human trial. Kirchheimer, *et al.* ([5]) have previously shown that armadillos which respond after vaccination (heat-killed *M. leprae* suspended in Freund's incomplete adjuvant) with positive lepromin reactions, delayed-type hypersensitivity skin reactions and lymphocyte-blast transformation to *M. leprae* antigens, do not develop disseminated leprosy following infection.

However, there is another point of view which needs to be mentioned here. Kirchheimer and Sanchez ([7]) have shown that about 80% of armadillos are susceptible to infection with even as few as 10^3 *M. leprae*. Thus, in contrast to human beings, most armadillos are susceptible to leprosy. Therefore, caution must be exercised in concluding that a vaccine which is effective against leprosy in armadillos would also be effective in man. The susceptibility of human beings to leprosy is not a species characteristic such as it appears to be in nine-banded armadillos. In humans this susceptibility might result from a specific defect which affects only a few persons and might not be corrigible by vaccination. Further well-planned studies will be necessary to resolve these questions.

SUMMARY

In this preliminary report the histopathological appearance of the lepromin reaction in armadillos was correlated with their response to infection with *M. leprae*. Three different types of lepromin response were described, namely the lepromatous, the borderline, and the tuberculoid lepromin reactions. It was found that 10 out of the 11 animals with a lepromatous lepromin reaction and the one animal with a borderline lepromin reaction developed disseminated disease. The two with a tuberculoid lepromin reaction and one of the 11 with a lepromatous lepromin reaction failed to develop leprosy. It is suggested that by using the lepromin response it is possible to assess, to a great extent, the susceptibility of armadillos to infection by *M. leprae*. The pros and cons of using the armadillo as an animal model for a vaccine trial are briefly discussed.

RESUMEN

En este informe preliminar, se correlaciona la apariencia histopatológica de la reacción a la lepromina en los armadillos con su respuesta a la infección con el *M. leprae*. Se describen tres tipos diferentes de respuesta a la lepromina; lepromatosa, intermedia (borderline), y tuberculoide. Diez de los once animales con una reacción lepromatosa a la lepromina, y el único animal con reacción del tipo intermedio a la misma, desarrollaron una enfermedad diseminada. Los dos animales con una reacción del tipo tuberculoide y uno de los once con reacción lepromatosa no desarrollaron lepra. Se sugiere que valorando la respuesta a la lepromina es posible establecer, con gran seguridad, la susceptibilidad de los armadillos a la infección por el *M. leprae*. También se discuten brevemente los pros y los contras del uso del armadillo como modelo animal para el estudio de vacunas contra la lepra.

RÉSUMÉ

Dans ce rapport préliminaire, on étudie la corrélation entre les aspects histopathologiques de la réaction à la lépromine chez le tatou, avec la réponse de ces animaux à l'infection par *M. leprae*. Trois types différents de réponse à la lépromine sont décrits, à savoir les réactions lépromateuse, dimorphe, et tuberculoïde. On a observé que dix des onze animaux présentant une réaction lépromateuse à la lépromine, de même qu'un animal avec une réaction à la lépromine de type

dimorphe, ont développé une maladie disséminée. Les deux animaux présentant une réaction à la lépromine de type tuberculoïde, ainsi qu'un des onze animaux avec une réaction à la lépromine de type lépromateux, n'ont pas développé la lèpre. On suggère dès lors que l'utilisation de la réponse à la lépromine rend possible d'estimer, dans une grande mesure, la susceptibilité des tatous à l'infection par *M. leprae*. Les avantages et les désavantages du tatou comme modèle animal pour un essai de vaccin sont discutés brièvement.

Acknowledgments. We are grateful to Mrs. Mary M. Jackson for secretarial help and to Mr. Gregory T. McCormick for making the histological sections.

REFERENCES

1. Azulay, R. D., Cesar de Andrade, L. M., Silva, C., Rabelo Netto, A. V., Azulay, J. D., Neves, R. G. and Alonso, A. M. Comparison of the macroscopic readings and microscopic findings of the lepromin reaction. Int. J. Lepr. **28** (1960) 38–43.
2. Dharmendra and Chatterjee, B. R. Prognostic value of the lepromin test in contacts of leprosy cases. Lepr. India **27** (1978) 149.
3. Kirchheimer, W. F. and Storrs, E. E. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. I. Report of lepromatoid leprosy in an experimentally infected armadillo. Int. J. Lepr. **39** (1971) 692–701.
4. Kirchheimer, W. F. and Storrs, E. E. Leprosy in experimentally infected armadillos. Seventh Annual Leprosy Research Conference, U.S.-Japan Cooperative Medical Science Program, Menlo Park, California, U.S.A., 1972. Int. J. Lepr. **40** (1972) 212–213.
5. Kirchheimer, W. F., Sanchez, R. M. and Shannon, E. J. Effects of specific vaccine on cell-mediated immunity of armadillos *M. leprae*. Int. J. Lepr. **46** (1978) 353–357.
6. Kirchheimer, W. F. Advances in biomedical leprosy research. Jap. J. Lepr. **49** (1980) 209–219.
7. Kirchheimer, W. F. and Sanchez, R. M. Intraspecies differences of resistance against leprosy in nine-banded armadillos. Lepr. India **53** (1981) 525–530.
8. Mitsuda, K. On the value of skin reaction to a suspension of leprous nodules. Jap. Derm. Urol. **19** (1919) 697–708. Reprinted in Int. J. Lepr. **21** (1953) 347–348.
9. Mohamed Ali, P. A study of conjugal leprosy. Int. J. Lepr. **33** (1965) 223–227.
10. Nagar, K. Histological findings at the site of Mitsuda reaction. Int. J. Lepr. **8** (1940) 132.
11. Neeves, R. G. A cytological study of lepromin test. Int. J. Lepr. **31** (1963) 577.
12. Nolasco, J. O. The lepromin test in lepra reaction—histology of reaction lesions and persistence of infected bacilli. Int. J. Lepr. **8** (1940) 285–298.
13. Rodriguez, J. N. Observation in lepromin (Mitsuda) reaction. Int. J. Lepr. **6** (1938) 11–32.
14. Skinsnes, O. K. The immunological spectrum of leprosy. In: *Leprosy in Theory and Practice*, 2nd ed., Cochrane, R. G. and Davey, T. F., eds. Bristol, England: John Wright & Sons, 1964, p. 156.
15. Thomas, J., Joseph, M., Ramanjam, K., Chacko, C. J. G. and Job, C. K. The histology of Mitsuda reaction and its significance. Lepr. Rev. **51** (1980) 329–339.
16. Turk, J. L. Cell mediated immunological process in leprosy. Bull. WHO **41** (1969) 779–792.