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Infection of Other Experimental Animals with *Mycobacterium leprae*

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Mycobacterium leprae multiply to a limited degree in immunologically intact rodents of several species, and to a greater degree in immunologically disabled rodents and in the armadillo.

Although multiplication of *M. leprae* in the foot pads and ears of rodents other than the mouse has not been as extensively nor as carefully studied as that in the foot pads of mice, there appear to be no important differences of bacterial multiplication among rodent species. Thus, the same "ceiling" to multiplication of *M. leprae* is encountered in the immunologically intact rat ([6, 8]), hamster ([2, 12, 15]), gerbil ([12]), and mystromys ([2]) as in intact mice. This is so despite the much larger mass of the foot pad of the rat than of the mouse.

The congenitally athymic rat. In the normal rat, *M. leprae* multiplies much as it does in the normal mouse ([6, 8]). Similarly, in the rat, as in the mouse, non-specific suppression of T-cell-mediated responses, by neonatal thymectomy, for example, permits a more generalized infection with *M. leprae,* with heavy infiltration of the cooler tissues ([6]).

The congenitally athymic rat, first described in 1978 ([5]), shares with the congenitally athymic nude mouse many of the characteristics associated with congenital athymia, including acceptance of skin and tumor xenografts, lack of response of splenic lymphocytes to T-cell mitogens, and enhanced susceptibility to a number of infectious agents. At the same time, the congenitally athymic rat survives much better under conventional conditions of husbandry than does the nude mouse, and is better able to control the multiplication of *Listeria monocytogenes* and *Toxoplasma gondii* (M. J. Colston, unpublished data) and the growth of tumor implants ([3]).

After inoculation of *M. leprae* into the foot pads of athymic rats, the organisms multiplied to a higher level than in neonatally thymectomized or euthymic rats ([4]). However, the number of organisms never exceeded 10^9 per foot pad. Intravenous inoculation of 10^7 organisms resulted in a disseminated infection that involved the tail, ears, foot pads, and snout. But after about one year, the number of *M. leprae* at each of these sites appeared to reach a plateau of about 10^8 ([4]). Histopathologic examination revealed the presence of lesions resembling those characteristic of lepromatous leprosy in man, with foamy macrophages and large numbers of organisms present in globi ([4]).

Thus, athymic rats are more susceptible to infection with *M. leprae* than are thymectomized-irradiated mice or neonatally thymectomized rats, but less so than are nude mice. Morphologic evidence suggests that the ability of the congenitally athymic rat to limit infection with *M. leprae* depends at least in part on the presence of a larger than normal number of activated macrophages. Activated macrophages or other non-T-cell-mediated cellular mechanisms would also explain the relative resistance of this animal to the growth of tumor implants.

The armadillo. Inoculation with *M. leprae* of the nine-banded armadillo (*Dasypus novemcinctus* Linn.), initiallly selected because of the low core temperature (30–36°C) characteristic of this animal species, has resulted in generalized progressive disease in a large fraction of the animals ([9]), and subsequent studies ([1]) have demonstrated the susceptibility to *M. leprae* infection of some armadillo species, and the apparent insusceptibility of others. Armadillos cannot be bred in captivity, but must be obtained from the wild in the Western Hemisphere, to which they are native. Before inoculation

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with *M. leprae,* they must be first quarantined for 3–4 months in a holding area, in which they are isolated from contact with wild armadillos, rodents and arthropods. During this time, they must be examined by serological and histopathological methods to exclude as far as possible the presence of mycobacterial infections. Not all nine-banded armadillos are susceptible to *M. leprae,* however. As many as 60% develop progressive and heavy systemic infection 12–24 months after intravenous inoculation of 10^8 *M. leprae.*

Other species. Infection with *M. leprae* of a few other animal species is of interest. Experimental leprosy of the European hedgehog has been reported ([1, 10]). A few studies of non-human primates have been described. Gunders, working in Liberia, reported ([7]) the first successful experimental infection of a chimpanzee, and Waters and his co-workers ([14]) described successful experimental infection of a gibbon in Malaysia. Finally, successful inoculation of rhesus, mangabey, and African green monkeys has recently been reported ([11, 13]).

Discussion. The experimental species to be inoculated depends upon the purpose of the work. Immunologically normal mice suffice for detecting viable *M. leprae* in proportions $\geq 1{:}10^3$, and for drug screening, and there appears no advantage to the use of some larger species, that is only more expensive to breed and maintain. To detect persisting *M. leprae,* the thymectomized-irradiated mouse and the neonatally thymectomized rat appear to offer the best compromise between the ability to detect small proportions of viable organisms and ease of maintenance; smaller proportions can be detected in nude mice, but at a disproportionate cost in terms of the effort required to work with them. For experimental chemotherapeutic studies, on the other hand, which require a greater degree of immunosuppression, there appears no choice but to employ nude mice.

With respect to the other experimental animals mentioned, the most noteworthy is the armadillo. This species has provided the large numbers of *M. leprae* required for vaccine development, and can provide the quantities of organisms required for biochemical studies and as a source of *M. leprae* DNA. It has been suggested that the *M. leprae*-infected armadillo might serve as an excellent model of the patient for experimental chemotherapeutic studies; however, one wonders if chemotherapeutic studies in the armadillo will present any advantage over clinical trials, in terms of expense and the time required for such studies.

REFERENCES

1. Amescua, M. E., Balina, L. M., Binford, C. H., Convit, J. C., Fieldsteel, A. H., Innami, S., Kirchheimer, W. F., Klingmueller, G., Kohsaka, K., Lew, J., Nakamura, K., Opromolla, D., Rees, R. J. W., Storrs, E. E. and Walsh, G. P. Workshop on experimental leprosy. Lepr. Rev. **50** (1979) 51.
2. Binford, C. H. The transmission of *M. leprae* to animals to find an experimental model. Int. J. Lepr. **36** (1968) 599.
3. Colston, M. J., Fieldsteel, A. H. and Dawson, P. J. Growth and regression of human tumor cell lines in congenitally athymic rates (*rnu/rnu*). J. Natl. Cancer Inst. **66** (1981) 843.
4. Dawson, P. J., Colston, M. J. and Fieldsteel, A. H. Infection of the congenitally athymic rat with *Mycobacterium leprae.* Int. J. Lepr. **51** (1983) 336.
5. Festing, M. F. W., May, D., Connors, T. A., Lovell, D. and Sparrow, S. An athymic nude mutation in the rat. Nature **274** (1978) 365.
6. Fieldsteel, A. H. and McIntosh, A. H. Effect of neonatal thymectomy and antithymocytic serum on susceptibility of rats to *Mycobacterium leprae* infection. Proc. Soc. Exper. Biol. Med. **138** (1971) 408.
7. Gunders, A. E. Progressive experimental infection with *Mycobacterium leprae* in a chimpanzee. A preliminary report. J. Trop. Med. Hyg. **61** (1958) 228.
8. Hilson, G. R. F. Observations on the inoculation of *M. leprae* in the foot pad of the white rat. Int. J. Lepr. **33** (1965) 662.
9. Kirchheimer, W. F. and Storrs, E. E. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. 1. Report of lepromatoid leprosy in an experimentally infected armadillo. Int. J. Lepr. **39** (1971) 693.
10. Klingmueller, G. and Sobich, E. Ubertragung menschlicher Leprabakterien auf der Igel. Naturwissenschaften **64** (1977) 5645.
11. Meyers, W. M., Walsh, G. P., Brown, H. L., Fukunishi, Y., Binford, C. H., Gerone, P. J. and Wolf, R. H. Naturally-acquired leprosy in a mangabey monkey (*Cercocebus* sp.). Int. J. Lepr. **48** (1980) 495.
12. Shepard, C. C. The experimental disease that fol-

lows the injection of human leprosy bacilli into foot pads of mice. J. Exper. Med. **112** (1960) 445.
13. WALSH, G. P., MEYERS, W. M., BINFORD, C. H., BROWN, H. L., WOLF, R. H., GORMUS, B. J., MARTIN, L. N., BASKIN, G. B. and GERONE, P. J. Experimental leprosy in the rhesus monkey and the African green monkey. Int. J. Lepr. **52** (1984) 735.
14. WATERS, M. F. R., ISA, B. B. H., REES, R. J. W. and MCDOUGALL, A. C. Experimental leprosy in the white-handed gibbon (*Hylobatus lar*): successful inoculation with leprosy bacilli of human origin. Brit. J. Exper. Pathol. **59** (1978) 551.
15. WATERS, M. F. R. and NIVEN, J. S. F. Experimental infection of the ear and foot pad of the golden hamster with *Mycobacterium leprae*. Brit. J. Exper. Pathol. **47** (1966) 86.