Overview of Animal Models

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Overview

Animal models have made important contributions to the understanding and treatment of many human diseases. For example, an understanding of the pathogenesis of tuberculosis stemmed largely from observations of the infection in animals. Leprosy, caused by *Mycobacterium leprae* and *Mycobacterium lepromatosis*, is the second most common mycobacterial infection in man. It is both an infectious disease with unique immunological features and a disease of the peripheral nerves. Historically, an improved understanding of the leprosy pathogenesis and the development of effective therapies have been slowed by the absence of an experimental animal model.

In 1873, Gerhard Armauer Hansen in Norway described the leprosy bacillus, opening the door to investigations of leprosy. Correspondingly, efforts began to establish *in vitro* culture methods and to identify animal models of the disease. Indeed, to prove to his many detractors that the rods he described in the tissues of human leprosy patients were the cause of leprosy, Hansen himself attempted *in vitro* cultivation and the identification of animal models. Despite his and many others' herculean efforts over nearly 150 years, the bacillus has never been successfully cultured in the laboratory. That single fact has intensified the emphasis placed on finding an animal model that can replicate the spectrum of features in this human disease, especially in lepromatous leprosy (LL). The need to replicate the features of LL is particularly important because the control of leprosy primarily depends on the detection and treatment of LL (see Chapter 1.2).

The ideal experimental model of leprosy would be an immunologically intact animal that exhibits the clinical spectrum of the disease, especially LL (see Chapter 2.4); has reactional episodes, i.e., erythema nodosum leprosum and reversal reactions (see Chapter 2.2); and develops peripheral neuritis with disability (see Chapter 2.5). Species phylogenetically similar to humans offer the

best hope of meeting these conditions. The animal must be readily adaptable to a laboratory setting, live long enough to allow the natural course of the infection, and cost a reasonable amount.

The long delay in developing appropriate animal models of leprosy was due in part to the lack of an underlying rationale in early experiments to infect animals. Then, building upon an observation by Virchow in 1863 (1) that human leprosy lesion sites tended to be exposed to air, such as the anterior eye and the ear, several investigators in the late 1950s, including Binford, hypothesized that lesions were present not only on sites exposed to air but also on cooler portions of the body (2). In 1958, a report described an association between cool body sites and tissue damage, especially in the superficial nerves, in leprosy patients. In 1959 and 1965, in support of this hypothesis, several investigators described the successful transmission of leprosy to the ears and testes of hamsters (3). This success led the way to the development of two important animal models, both derived directly from the hypothesis of selective growth in cooler anatomic sites, that made possible many of the advances in subsequent years: the transmission of leprosy to the mouse footpad (*Mus musculus*) and the inoculation of the nine-banded armadillo (*Dasypus novemcinctus*; body temperature 32–35° C) with *M. leprae*, both described in more detail below (4, 5). See also Chapters 10.2 and 10.3.

Early on, some 30 species of animals, including guinea pigs, hamsters, rabbits, and rats, were inoculated with leprosy bacilli using a wide variety of methods (6). At best, several investigators obtained limited or evanescent disease. In addition, successful transmission claims were frequently misinterpreted. For example, one investigator who claimed to induce lepromin reactions in monkeys believed them to be active lepromatous lesions (7). However, lepromin is inactivated *M. leprae*, used as a skin test agent to predict susceptibility to leprosy. Another did not understand the long persistence of dead carcasses of *M. leprae* in inoculated hamsters, incorrectly reporting the successful experimental reproduction of leprosy in a laboratory animal.

In a landmark breakthrough around 1960, it was established that most types of immunologically intact laboratory mice develop localized infection after footpad or intradermal inoculation with *M. leprae*, the former of which became commonly used as an anti-leprosy drug screening model (4). Even though the disease in the footpad remained localized, it provided a basis for the study of the leprosy bacillus. Prior to this model, anti-leprosy drugs could only be screened and evaluated in leprosy patients. Thus, in addition to improving time, expense, and precision, this breakthrough offered a more ethical method of screening for new drugs.

There are several ways to assess a drug in the mouse footpad model, some reasonably complicated, for example, assessing whether a drug is bacteriostatic or bactericidal. In general, however, mouse footpads were injected with *M. leprae*, and the mouse was then given the drug of interest. The footpads were harvested and the acid-fast bacilli smeared on glass slides, stained using the Fite-Faraco method, and then painstakingly counted. New staining and molecular biology techniques that can better quantify *M. leprae* may be useful in overcoming human counting limitations (see Chapter 5.3).

Nonhuman reservoirs of leprosy are now well-known. In the 1970s, investigators in the Southeastern part of the United States reported that the nine-banded armadillo, though phylogenetically distant from humans, is susceptible to *M. leprae* and develops cutaneous and visceral lesions of LL after experimental administration of *M. leprae* or through naturally acquired leprosy. Naturally acquired leprosy in armadillos was first reported in Louisiana in 1974 and, in some areas, up to 60% of armadillos were infected (see Chapter 10.2) (8, 9, 10). Later, more sophisticated studies in 2011 sand 2015 showed that armadillos with naturally acquired disease were infected naturally long before the 1974 report, silencing detractors (11, 12). The 2011 report and several others corroborate the transmission of naturally acquired leprosy in armadillos to humans (13, 14, 15).

A reliable non-human primate model of human leprosy that developed the spectrum of features in human disease, especially neuritis and nerve-damaging reactions that lead to disability, would be useful for improving our understanding of the leprosy pathogenesis. The first adequately documented instance of disseminated experimental disease was in the 1950s in a chimpanzee, which was inoculated in the skin, nerve, peritoneal space, and bloodstream (16). A skin biopsy specimen housed at the Armed Forces of Institute of Pathology (AFIP) shows active borderline disease.

In the late 1980s, scattered reports of naturally occurring leprosy in chimpanzees (*Pan troglodytes*) and sooty mangabey monkeys (*Cercocebus atys*) triggered efforts to develop non-human primate models of leprosy. The sooty mangabey monkey, from West Africa, seemed most susceptible to leprosy, either naturally acquired or experimentally infected, but was not readily available. Recently, indigenous leprosy of multiple genotypes has been detected in several chimpanzee and monkey populations in Africa and South America (17, 18). Earlier studies found indigenous leprosy in monkeys in West Africa and elsewhere (19, 20, 21).

The discoveries of a naturally acquired leprosy-like disease in chimpanzees, a mangabey monkey from West Africa, and a cynomolgus macaque (*Macaca fascicularis*) from the Philippines further underscore the notion that leprosy can be zoonotic (20, 21, 22, 23). Leprosy in mangabey monkeys resembles human LL, with skin lesions, nerve palsy, immunological abnormalities, immune-mediated like reactions, and histological features. The chimpanzee and rhesus monkey (*Macaca mulatta*) seem susceptible to leprosy, but the rhesus monkey tends to develop tuberculoid (localized) disease only (24). Leprosy has been transmitted successfully from the mangabey monkey to other mangabeys, rhesus monkeys, and African green monkeys (*Chlorocebus aethiops*). However, leprosy transmission from naturally infected mangabey monkeys or chimpanzees to humans has not been reported.

A report published in 1941 vaguely describes the experimental inoculation of *M. leprae* into one cynomolgus monkey, and there is one report of naturally acquired borderline lepromatous leprosy (BL) in a captive Philippine cynomolgus monkey (25). However, a long-term, large follow-up study in the Philippines showed that captive bred cynomolgus monkeys, much like rhesus monkeys, are not especially susceptible to experimental infection (25). Research using these various primate models has been limited due to the high cost of and difficulty in obtaining and maintaining them.

Establishing animal models that replicate the full spectrum of human LL has been extraordinarily difficult. However, despite great cuts in funding, work continues in some laboratories. Whereas some models described above such as the nine-banded armadillo and mouse footpad, including nude mice, offer certain advantages such as studying leprosy immunology and even pathogenesis such as nerve damage (6, 26), no single model develops all the features of human LL (27, 28). An important feature that current models lack is the development of immune-mediated reactions, as these cause the majority of long-term disabilities in humans.

References

1.   **Binford CH**. 1953. Virchow's description of the lepra cell. Int J Lepr **21**:372–373.

2.   **Binford CH**. 1956. Comprehensive program for the inoculation of human leprosy into laboratory animals. US Public Health Reports **71**:995–996.

3.   **Binford CH**. 1959. Histiocytic granulomatous mycobacterial lesions produced in the golden hamster (*Cricetus auratus*) inoculated with human leprosy; negative results in ten experiments using other animals. Int J Lepr **26**:318–324.

4.   **Shepard CC**. 1960. The experimental disease that follows the injection of human leprosy into footpads of mice. J Exp Med **112**:445–454.

5.   **Storrs EE, Walsh GP, Burchfield HP, Binford CH**. 1974. Leprosy in the armadillo: new model for biomedical research. Science **183**:851–852.

6.   **Adams LB, Pena MT, Sharma R, Hagge DA, Schurr E, Truman RW**. 2012. Insights from animal models on the immunogenetics of leprosy: a review. Mem Inst Oswaldo Cruz **107(Suppl 1)**:197–208.

7.   **Soule MH, McKinley EB**. 1932. Cultivation of *B. leprae* with experimental lesions in monkeys Am J Trop Med **12**:1–36.

8.   **Walsh GP, Storrs EE, Burchfield HP, Cotrell EH, Vidrine MF, Binford CH**. 1975. Leprosy-like disease occurring naturally in armadillos. J Reticuloendothel Soc **18**:347–351.

9.   **Binford CH, Meyers WM, Walsh GP, Storrs EE, Brown HL**. 1977. Naturally acquired leprosy-like disease in the nine-banded armadillo (*Dasypus novemcinctus*): histopathologic and microbiologic studies of tissues. J Reticuloendothel Soc **22**:377–388.

10.   **Walsh GP, Storrs EE, Meyers W, Binford CH**. 1977. Naturally acquired leprosy-like disease in the nine-banded armadillo (*Dasypus novemcinctus*): recent epizootiologic findings. J Reticuloendothel Soc **22**:363–367.

11. **Truman RW, Singh P, Sharma R, Busso P, Rougemont J, Paniz-Mondolfi A, Kapopou-lou A, Brisse S, Scollard DM, Gillis TP, Cole ST**. 2011. Probable zoonotic leprosy in the southern United States. N Engl J Med **364**:1626–1633.

12. **Sharma R, Singh P, Loughry WJ, Lockhart JM, Inman WB, Duthie MS, Pena MT, Mar-cos LA, Scollard DM, Cole ST, Truman RW**. 2015. Zoonotic leprosy in the southeastern United States. Emerg Infect Dis **21**:2127–2134.

13. **Lane JE, Walsh DS, Meyers WM, Klassen-Fischer MK, Kent DE, Cohen DJ**. 2006. Borderline tuberculoid leprosy in a woman from the state of Georgia with armadillo exposure. J Am Acad Dermatol **55**:714–716.

14. **Lane JE, Meyers WM, Walsh DS**. 2009. Armadillos as a source of leprosy infection in the southeast. South Med J **102**:113–114.

15. **Nolen L, Haberling D, Scollard D, Truman R, Rodriguez-Lainz A, Blum L, Blaney D**. 2014. Incidence of Hansen's Disease—United States, 1994–2011. MMWR Morb Mor-tal Wkly Rep **63**:969–972.

16. **Gunders AE**. 1958. Progressive experimental infection with Mycobacterium leprae in a chimpanzee; a preliminary report. J Trop Med Hyg **61**:228–230.

17. **Hocking KJ, Mubemba B, Avanzi C, Pleh K, Düx A, Bersacola E, Bessa J, Ramon M, Metzger S, Patrono LV, Jaffe JE, Benjak A, Bonneaud C, Busso P, Couacy-Hymann E, Gado M, Gagneux S, Johnson RC, Kodio M, Lynton-Jenkins J, Morozova I, Mätz-Rens-ing K, Regalla A, Said AR, Schuenemann VJ, Sow SO, Spencer JS, Ulrich M, Zoubi H, Cole ST, Wittig RM, Calvignac-Spencer S, Leendertz FH.** 2020. Leprosy in wild chim-panzees. bioRxiv https://doi.org/10.1101/2020.11.10.374371.

18. **Honap TP, Pfister LA, Housman G, Mills S, Tarara RP, Suzuki K, Cuozzo FP, Sauther ML, Rosenberg MS, Stone AC**. 2018. *Mycobacterium leprae* genomes from naturally infected nonhuman primates. PLoS Negl Trop Dis **12**:e0006190.

19. **Fukunishi Y, Meyers WM, Binford CH, Walsh GP, Johnson FB, Gerone PJ, Wolf RH, Gormus BJ, Martin LN**. 1984. Electron microscopic study of leprosy in a mangabey monkey (natural infection). Int J Lepr Other Mycobact Dis **52**:203–207.

20. **Gormus BJ, Xu K, Baskin GB, Martin LN, Bohm RP, Blanchard JL, Mack PA, Ratterree MS, McClure HM, Meyers WM, Walsh GP**. 1995. Experimental leprosy in monkeys. I. Sooty mangabey monkeys: transmission, susceptibility, clinical and pathological find-ings. Lepr Rev **66**:96–104.

21. **Gormus BJ, Xu K, Cho SN, Baskin GB, Bohm RP, Martin LN, Blanchard JL, Mack PA, Ratterree MS, Meyers WM, Walsh GP**. 1995. Experimental leprosy in monkeys. II.

Longitudinal serological observations in sooty mangabey monkeys. Lepr Rev **66**:105–125.

22. **Meyers WM, Walsh GP, Brown HL, Binford CH, Imes GD, Jr., Hadfield TL, Schlagel CJ, Fukunishi Y, Gerone PJ, Wolf RH, Gormus BJ, Martin LN, Harboe M, Imaeda T**. 1985. Leprosy in a mangabey monkey—naturally acquired infection. Int J Lepr Other Mycobact Dis **53**:1–14.

23. **Walsh GP, Meyers WM, Binford CH, Gormus BJ, Baskin GB, Wolf RH, Gerone PJ**. 1988. Leprosy as a zoonosis: an update. Acta Leprol **6**:51–60.

24. **Gormus BJ, Xu K, Baskin GB, Martin LN, Bohm RP, Jr., Blanchard JL, Mack PA, Ratterree MS, Meyers WM, Walsh GP**. 1998. Experimental leprosy in rhesus monkeys: transmission, susceptibility, clinical and immunological findings. Lepr Rev **69**:235–245.

25. **Walsh GP, Dela Cruz EC, Abalos RM, Tan EV, Fajardo TT, Villahermosa LG, Cellona RV, Balagon MV, White VA, Saunderson PR, Walsh DS**. 2012. Limited susceptibility of cynomolgus monkeys (*Macaca fascicularis*) to leprosy after experimental administration of *Mycobacterium leprae*. Am J Trop Med Hyg **87**:327–336.

26. **Scollard DM, Truman RW, Ebenezer GJ**. 2015. Mechanisms of nerve injury in leprosy. Clin Dermatol **33**:46–54.

27. **Oliveira I, Deps PD, Antunes J**. 2019. Armadillos and leprosy: from infection to biological model. Rev Inst Med Trop Sao Paulo **61**:e44.

28. **Vilani-Moreno FR, Barbosa A, Sartori BGC, Diório SM, Silva S, Rosa PS, de Faria Fernandes Belone A, Soares CT, Lauris JRP, Pedrini SCB**. 2019. Murine experimental leprosy: evaluation of immune response by analysis of peritoneal lavage cells and footpad histopathology. Int J Exp Pathol **100**:161–174.