ANIMAL MODELS IN LEPROSY

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INTRODUCTION

The cultivation of human pathogens in experimental animals have made enormous contribution to the understanding and treatment of many diseases of humans. Even though the *Mycobacterium leprae* was one of the first human bacterial pathogens to be identified, has not been cultivated in vitro till 1960. The long delay in developing appropriate animal models of leprosy, hindered the progress in leprosy research. The long search for an animal model for leprosy has involved almost 30 species of animals and almost as many protocols as research.

Key words: Leprosy, animal models, Hansen's disease, armadillo, Foot pad infection, *Mycobacterium leprae*.

HISTORY

When Hansen demonstrated the rod shaped *Mycobacterium leprae* on 20th February 1873, leprosy became one of the first diseases to be linked with a microbial pathogen, and *M. leprae* was one of the first human bacterial pathogens to be identified. Thus he opened the door to experimental leprosy. But he was failed to transmit the infection to a very wide range of animal species. The first unequivocally successful and reproducible transmission of a limited infection to animals was by "Shepard" (1960) in the mouse footpad. Likewise a reproducible but

progressive *M. leprae* infection resulted in the nine-banded armadillos inoculated intravenously with *M. leprae*. The first attempt to inoculate the nine-banded armadillo was described by Kirchheimer and Storers in 1971. These two animals are the chief animal models in experimental leprosy. The transmission of leprosy to the mouse footpad, and the inoculation of the armadillo were based directly on the hypothesis of selective growth of M. leprae in cooler anatomic sites, which was postulated by Binford in 1956. These two animal models have made possible many of the remarkable advances in recent years in leprosy research and patient management.

ANIMAL MODELS

The animals used for experimental studies can be described under three groups: Rodents, Armadillos and non-human primates.

RODENTS Normal mice: In 1960 Shepard succeeded in inoculation and multiplication of *M. laprae* in the foot pads of CFW mice. It was a breakthrough in leprosy research.

The standard foot pad infection in mice results from a subcutaneous inoculation into the plantar space of the hind footpad with 5x10^3 to I0^4 acid fast Bacilli organisms in a volume of 0.03m1.

Which yields up to 10^6 AFB/footpad by 180 days. Total number of stainable bacilli remains constant at approximately 10^6 for about 1 year and then the number of viable organisms decrease rapidly after the plateau is reached. The minimal infective dose of M. leprae for normal mouse footpad is estimated at 1—10 living organism. BAL B/c, CBA, CFW and DBA stains of mice seems to produce higher levels of infection than the most other stains of mice such as C57 BL. Histological changes appear as early as 3 months post infection and consist of small infiltration of macrophages and lymphocytes. Some of the macrophages contain clusters of acid fast bacilli, and may show epitheloid cell changes and poorly organized granulomas. Sometimes there is bacillary invasion of the perineuria and nerves. Systemic spread does not develop.

Nude mice: These athymic animals were first reported in leprosy studies by Dr Kohsaka, Dr. Colston and colleagues in 1976. The nude mice is an alternative to using thymectomised mouse like the T. 900 R mouse, the nude mouse will permit multiplication of M. leprae from footpad inoculation. This is accompanied by gross local swelling as well as haematogenous spread to other cooler sites. After 18 months of inoculation there was significant liver and spleen involvement.

Beige mice: These immunologically deficient mice have been used for a number of years in biomedical researches but are new in leprosy studies Dr. Dhople reported that M. leprae multiplication in spleen and liver could be detected as early as 4 months in mice inoculated intra peritoneal or intra-venous. Statistically significant enhancement of growth of M. leprae in foot pads of beige compared to normal BAL B/c mice was also observed. Dr. Dhople also found the model to be suitable for chemotherapy studies.

Thymectomise -irradiated mice: This animal model was developed and extensively applied to study the growth of M. Ieprae and immunopathology of leprosy by "Dr. Rees" and colleagues in 1966. Rees reported on the pathogenesis of leprosy in CBA mice that had undergone.

Thymectomy followed by whole body irradiation of 9 Gy (900 rad), and subsequent inoculation of *M. leprae* into the foot pads and ears. After nine months the infected foot pad is swollen, there was haematogenons spread to other cooler peripheral parts—i.e. other foot pads, ears, nose and tail, often associated with local swelling.

Neonatally thymectomised lewis Rats (NTLR): This immunodeficient animal has also been used as a model for studies in leprosy, and it is very much like the T-900 R mouse in its response to infection with M. leprae. Moreover it has a better survival, but unfortunately there is a very marked variation in the degree of immune-deficiency between animals for unknown reason.

Rats and other Rodents: Although multiplication of M. leprae in the foot—pads and ears of rodents other than the mouse has not been so extensively studied, the limited pattern of growth and plateau effect are similar to those in the mouse. The rodents studied include the Rat, Hamster, Gerbil, and Mystromys. Foot pad infection is never associated with local swelling in normal rodents.

M. leprae locally in the skin or intravenously. Disseminated leprosy was observed in skin, bone marrow, liver, spleen, lymph nodes, lung, meninges and eyes. Additionally leprotic pneumonitis, leprotic meningitis and oesophagcal involvement were described. The degree of lepromatous leprosy in armadillo is thus more severe than in man. Histopathologic examination reveals heavy infiltration of M. leprae-laden macrophages in lymph nodes, liver, spleen, bone marrow and eye, with frequent invasion of the central nervous system and lungs. **Other armadillo species:** Seven-banded armadillos and eight-banded armadillos were also found to develop leprosy lesions.

NON-HUMAN PRIMATES

Of the many other different animal models for experimental leprosy, the monkeys or apes are the most ideal models. The advantages of these models are most biologically active reagents employed in the laboratory are highly cross—reactive between humans and the higher non—human primates.

Chimpanzees: In 1958 Gunders described disseminated experimental leprosy in one of two inoculated chimpanzees. This is probably the first well-documented transmission of disseminated leprosy to a non-human primate. After 11 months of inoculation he observed nodules on ear, hands, feet and legs with large area of depigmentation. After 3 more months the nodules had regressed, leaving the areas of depigmentation. Biopsy of skin showed active borderline leprosy.

White handed Gibbon: Waters *et al* (1978), reported transmission of leprosy to a single white-handed Gibbon, with an observation period of nearly 15years. The inoculation was given by intravenously, intra peritoneal routes, into the testis, ears and ulnar nerve.

Sooty mangabey monkeys: The discovery of naturally acquired leprosy in a sooty mangabey monkey in 1979. Sparked interest in monkeys as model of leprosy. It is likely the first mangabey acquired the disease and was the source of infection for the second animal with which it was caged for a number of months. This appears to be the first case of monkey-to-monkey transmission. Experiment leprosy studies in sooty mangabey monkeys revealed that this species was very susceptible to leprosy.

Rhesus monkeys: Between 1980 and 1987, 38 rhesus monkeys were inoculated with M. leprae. Seven out of 38 i.e. 18% developed BL-LL leprosy. Incubation periods ranged from 2-47 months peripheral neuritis was a prominent feature. Whereas sooty mangabey monkeys tend to have multibacillary disease, the rhesus monkeys tend toward more resistant forms of leprosy.

African green monkeys: A total of 19 African green monkeys have been inoculated with M. leprae. Within 4-22 months there were nodules of multibacillary leprosy at inoculation sites and the nasal smear were AFB positive. Clinically, there was dissemination to non inoculated cutaneous sites in one animal. The post-mortem examination reveals advanced polyneuritic leprosy, interpreted as B.L. disease.

Cynomolgus monkeys: **I**n 1990 Walsh et al, described experimental studies in Philippine cynomolgus monkeys. They used 24 cynomolgus monkeys for the experimental leprosy. Out of this 24, four animals have developed the lesion of boarder line leprosy at the site of inoculation, within 3-9 months of period, but the lesions regressed soon after. There were strong antibody responses to PGL-1 beginning at 1-2 months. **Squirrel monkeys:** Three squirrel monkeys received M.leprae intravenously and intracutaneously. None of the animal showed clinical evidence of leprosy. Post-mortem examination also revealed no evidence of leprosy. **Tupaias (Tree shrews)**: Wang et al, in 1990 inoculated M. leprae into 13 tupaias, 9 animals intravenously and 4 animals in the foot pad. There was disseminated leprosy at 13-19 months post inoculation. Histologically there were lepromatous in filtrates with invasion of cutaneous nervous system.

APPLICATIONS OF ANIMAL MODELS

Animal inoculation of laboratory animals with M. leprae has been successfully applied for both laboratory and clinical research. Undoubtedly, the mouse takes pride of place in view of its availability, ease of handling and small size (25gm). On the other hand the armadillo in view of its large size (3-5kg) and natural susceptibility, take pride of place as the model for large scale laboratory production of M. leprae. At the same time non-human primates are the best long term animal models on the basis of studies on the pathogenesis of leprosy.

a) Screening of anti leprosy drugs: The M. leprae foot pad infection in normal mouse was, and continues to be, the most convenient and reliable animal model for screening new anti-leprosy drugs. There are 3 methods for screening drugs against M. leprae in mice.

1) Continuous method
2) Kinetic method
3) Proportional bactericidal method.

In the continuous method the drug is administrated from the time of inoculation and the non treated control mice are monitored until plateau number of AFB are reached. At this time counts from the treated animals are determined and compare with non treated. All the new drugs are screened by the continuous method. However the continuous method does not distinguish between a bactericidal and bacteriostatic activity of the drug. To make this important distinction, the drug should start 60 days after the inoculation and continued for 60-70 days, instead of giving the drug immediately after the inoculation. This method is called kinetic method. The time to reach plateau growth by the treated mice is compared with that by the untreated — if there is no delay the drug is inactive, if the delay is 60-70 days only it is bacteriostatic and if it is more than 70 days it is bacteriocidal. The proportional bactericidal method is also used to measure the bactericidal activity of a drug. In this procedure 10 fold dilutions of AFB are inoculated. The drug is administered from day 1 to 60. Footpad counts are carried out after 12 months of inoculation. The proportional bacteriocidal method cannot differentiate between inactive drugs and drugs that are purely bacteriostatic.

b) Detection of drug resistance: The drug resistance study is the most important chemotherapeutic application of the mouse model. It was first employed by Pettil and Recs in 1964 to establish secondary dapsone resistance. For this study continuous method is used. In 1976, *M. leprae* resistant to rifampicin were identified by Jacobsan and Hastings. Recently *M. leprae* resistant to dapsone, rifampicin and ofloxacin have been reported by Cambau *et al* (1997).

c) Studies on the pathogenesis of leprosy: In spite of very considerable contributions of the various mouse models for studying the early development of the pathogenesis of leprosy, their short life span of at the most 2 years, is in adequate for a longer term follow-up of a chronic infection such as leprosy. Furthermore the armadillo is also unsuitable as it is particularly susceptible and develops a fully fledged lepromatous infection within two years. Over the last few year limited studies on experimental leprosy infections in several non-human primates have been reported. They include successful leprosy infections in mangabey, African green monkeys and rhesus monkeys. The infections resemble the human disease of leprornatous and borderline types including nerve, skin and eye involvement, with gross evidence of nerve damage affecting the limb. These preliminary findings strongly suggest that non-human primates may well provide the best long term animal models for more complete studies on the pathogenesis of leprosy as seen in man.

Production of M. leprae: M. leprae remain a rare research resource. They cannot be cultivated on artificial media. In the absence of culture media, the major use of the armadillos is the production of M. leprae for various studies. One susceptible armadillo could harbour upto 10^11 to 10^12 M. leprae. Bacilli were extracted and purified without loss of viability according to a well-formulated protocol (WHO 1980) and supplied for various studies such as vaccine trials, immunologic, metabolic and genetic studies.

e) Viability of M. leprae: The foot-pad model had been used for detecting the viability of M. leprae. It was shown that M. leprae could survive in moist soil upto 46 days (Desikan and Sreevatsa 1979)

f) Vaccine studies: Normal mice vaccinated with heat-killed M. leprae were found resistant to subsequent M. leprae infection (Shepard et al 1983). Vaccination of mice with a soluble protein fraction from M. leprae was found to protect them from M. leprae infection (Gelber et al 1992).
g) Study of neuritis: Interneural injection of M. leprae into sciatic nerves produced a tuberculoid reaction in normal mice; and in immunosuppressed mice, a leprornatous response. However M. leprae failed to enter Schwann cells (Shetty 1993). It was found that Schwann cells of sciatic nerves in M. leprae, inoculated mice produced increased amounts of intraneural collagen (Singh et al 1998).
h) Generation time of M. lcprae: It is generally accepted that the doubling time of M. leprae is 12 to 13 days. In a study in nude mice by Hastings and Morales the doubling time of viable M. leprae was estimated to be as short as 26 hours (Hastings and Morales 1982).

CONCLUSION

There is obviously much attention paid historically to search for an animal model of leprosy. 140 years have yielded 3 species of armadillo, normal mouse, nude mice, rats, gibbon, rhesus monkeys, African green monkeys, chimpanzees etc, but more experimental studies are necessary to establish their utility. Today's technology will find the ultimate answer to this 140 yrs search for a suitable animal model for leprosy.

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