The contemporary relevance of the mouse foot pad model for cultivating *M. leprae*

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The fight against leprosy has been one of the major success stories of modern medicine. Even though *M. leprae*, the causative organism of leprosy, could not be grown in any acceptable *in-vitro* medium system, the microbiological need was fulfilled by the development of various animal models. This successful journey began with the report of limited multiplication of *M. leprae* in the footpads of ‘CFW’ mice. The findings of Shepard were confirmed by Rees. During the last nearly 50 years, the mouse foot pad (MFP) technique has been extensively evaluated and used. The inbred strains of BALB/C and CBA mice have been observed to be more susceptible to infection with *M. leprae* than the C57 BL and C3H mouse strains. The MFP technique has been used for viability determination, drug screening, testing immunoprophylactic agents, undertaking studies on determination of minimum inhibitory concentration (MIC) and minimum effective dose of various anti-leprotic compounds, and also for identification of *M. leprae*. The pattern of applications has changed from decade to decade; in the 1960s and 1970s it was used for viability determination, drug screening and experimental chemotherapy. The information derived from the mouse foot pad about the effect of single versus continuous administration in animals, and later in human beings, led to the designing of the WHO recommended multidrug treatment (MDT) for leprosy, comprising rifampicin, clofazimine and dapsone. These MDT regimens have been key in reducing the global leprosy problem. During the 1970s and 1980s, the focus of interest shifted to using this model for testing mycobacteria which could be used as immunotherapeutic and/or immunoprophylactic agents against leprosy. Identification of BCG, heat killed *M. leprae*, ICRC, *M. habana*, and *M. w.* are success stories which began with identification of the protective effect of these mycobacterial strains against leprosy in the mouse foot pad model. The MFP enabled the studies on the effectiveness of pulsed administration of rifampicin ofloxacin and minocycline against *M. leprae*. The growth characteristics and the involvement of nerve twigs in the mouse foot pad were used as a marker of identification of *M. leprae* from the 1960s to the 1990s. During the 1980s the greatest number of publications on different
applications of the MFP were seen with a subsequent decline in interest with the major successes in leprosy reduction achieved with MDT.

Major changes have occurred in the disease burden and clinical profile, and there has been a paradigm shift in the approach resulting from advances in molecular biology and genomics. Currently, the number of leprosy cases has declined in many countries, and highly bacillated cases (>3 + BI) are contributing a much smaller proportion (<10%) of the case load. There is intense debate going on all over the world as to whether countries and organizations should continue to support mouse foot pad laboratories which are currently being maintained by various governments or non-governmental organizations. In this context, it is important to revisit the original application areas of the MFP model:

i) Viability determination: The MFP was extensively used for assessing and monitoring the viability of leprosy bacilli for developing chemotherapy trials and also for studying the viability of *M. leprae* outside the human body. While this method was important for this research work, the MFP model now is of limited use. Firstly, most patients now have a low bacterial load and probably 90% do not have sufficient organisms i.e. about 5–10,000/MFP as AFB could be detected in 94% of slit smears in leprosy in a population survey from Ghantampur, North India (Kiran Katoch, unpublished data, ICMR Taskforce study report 2008). While some investigators have been able to grow *M. leprae* even from paucibacillary smear negative specimens, so far these are isolated reports. Secondly, there are now validated rapid alternatives for assessing *M. leprae* viability, methods such as ATP bioluminescence, PCR, RT-PCR and real time PCR targeting RNA. While techniques like ATP bioluminescence, which are based on estimation of bacterial ATP and require 100 live bacilli per specimen, molecular methods targeting *M. leprae* specific DNA, or RNA are able to detect less than 10 organisms. Such molecular methods are also useful to detect live *M. leprae* in the environment.

ii) Screening for Drug resistance: The MFP model was used extensively to determine primary and secondary resistance to anti leprosy drugs. While there are some reports of drug resistance to one or more drugs, the widespread use of MDT has significantly reduced the prevalence of drug resistance. Further, because of the requirement of a minimum size inoculum (5–10,000 organisms) for a normal MFP experiment with 5–6 mice/specimen, this technique *per se* has limited usefulness. While MFP has a role in defining the ‘viable unit’ for alternate *in vitro* assays for the purpose of detection of drug resistance, various molecular methods detecting DNA mutations have immense superiority in terms of feasibility, easiness of application and very high potential sensitivity. These methods are good for detecting rifampin resistance genes but limited in detecting resistance genes for other anti-leprosy drugs.

iii) Testing for immunophylactic agents: The MFP model has been useful in the identification of immunotherapeutic/immunophylactic agents against *M. leprae*. While this model is still being used for testing new vaccine candidates, it is questionable whether we are interested in identifying more agents at present. There is now only limited interest in this area.

iv) Studying Immunopathogenesis: During the last decade, many studies using MFP have been looking at the mechanisms of pathogenesis and protection in leprosy by dissecting the cells and pathways of the immune response, and also studying host-parasite interactions using genomic approaches. For investigators interested in
investigating the biology of *M. leprae* and host pathogen interactions, the MFP will continue to be useful.

v) **Experimental chemotherapy:** As the interest in identifying newer anti leprotic compounds has considerably decreased, this application will only be of interest to specialised research laboratories who continue to work on identifying newer antimycobacterial drugs that could be useful against a variety of infections including leprosy.

vi) **Identification of *M. leprae***: After the development of several probes and gene amplification methods for *M. leprae*, the need to grow *M. leprae* in MFP for identification purposes is merely of academic interest and no longer important.

To conclude, the usefulness and spectrum of application of the mouse foot pad has drastically changed over the years. While this model continues to be relevant for selective research purposes such as understanding the host parasite interaction, confirmation of activity of newer compounds and also experimental chemotherapy, other earlier applications, such as viability determination and identification of *M. leprae* for diagnostic and epidemiological purposes, can be more effectively done by alternate methods. For growing *M. leprae* in sufficient quantities, one needs immuno compromised animals and other animals like armadillos. All these issues have been extensively debated over the years. Leprosy is down but not yet gone. It is true that fewer researchers are interested in the applications of MFP but the need is still there. It would be important to maintain a few MFP laboratories in some selected countries/institutions to continue working on currently relevant aspects. At least one MFP laboratory in countries like India, Brazil, Nepal, Ethiopia, UK, The Netherlands and the USA should be supported with the support of national, international or non-governmental agencies.

References

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22 Also Special volumes/issues of International Journal of Leprosy 1987; Leprosy Review 1986, 57(suppl 3) and (Indian Journal of Leprosy 1991).