Rodent Models in Leprosy Research

Shannon M Lenz
IHRC, Inc

J Hunter Collins
US Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, National Hansen's Disease Programs

Ramanuj Lahiri
US Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, National Hansen's Disease Programs

Linda B Adams
US Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, National Hansen's Disease Programs

Overview

In 1960, Charles Shepard published his work on the growth of *Mycobacterium leprae* (see Chapter 5.1) in the footpads of immunocompetent Carworth Farms white mice (1). Although *M. leprae* was first associated with leprosy by Armauer Hansen nearly 90 years earlier, Shepard's passage of the bacilli in mouse footpads (MFP) was the first successful reproducible propagation of this organism. His was not, however, the first attempt. Since its discovery, numerous investigators have tried to grow *M. leprae* and reproduce leprosy in a variety of animal models, including common laboratory species such as rodents, rabbits, and monkeys and other mammals, birds, and

even cold-blooded animals [reviewed in (2)]. Most of these attempts were unsuccessful, some because of a natural resistance of the host species to infection, but others likely due to the poor viability of the human biopsy-derived inoculum and a lack of knowledge of the meticulous growth requirements of leprosy bacilli. Shepard and others (3, 4, 5) went on to confirm the utility of the MFP model for growing *M. leprae* and, for the first time, the establishment and maintenance of isolates of *M. leprae* was accomplished. Moreover, drug testing, detection of drug resistance, experimental vaccine evaluation, and immunological studies became feasible.

MICE

Mice (*Mus musculus*) are the experimental animals most commonly used in biomedical research. Due to their small size, they are relatively inexpensive to house and maintain. Mice are also prolific breeders with a relatively short gestation time, allowing for the rapid generation of large numbers of animals. There are hundreds of commercially available well-defined strains, including outbred, inbred, and genetically engineered mutants. Additionally, there is an ever-growing multitude of sophisticated mouse-specific biological reagents available that enable complex and detailed examination of physiological and immunological processes. Mice, in particular the MFP model, have been used extensively in leprosy research.

RATS

Brown rats (*Rattus norvegicus*) were the first mammals specifically domesticated for use in biomedical research in the 19th century (6). Rats have similar housing and maintenance requirements as mice, and they also have similarly short gestational periods and produce multiple litters in a year. However, while mice have been used across the entire spectrum of biomedical research, rats have been more frequently used to study human and animal physiology due to their larger size. Until the rat genome was sequenced in 2004 (7), it was more difficult for researchers to create transgenic rats, so their use in genetic studies lagged behind mice (8). There are now numerous inbred, outbred, and genetic mutant strains of rats commercially available. While useful as biomedical animal models, rats are not frequently used in leprosy research today.

SQUIRRELS

Recently, red squirrels (*Sciurus vulgaris*) have been identified as natural reservoirs of both *M. leprae* and *M. lepromatosis* (9). Interestingly, only *S. vulgaris* squirrels from the British Isles seem to be infected with resultant leprosy-like lesions. Schilling et al. (10) used PCR to test red squirrels from other parts of Europe, along with four other squirrel species from around the world. They found no evidence of either *M. leprae* or *M. lepromatosis* in any of the other squirrel species. While red squirrels may represent another potential leprosy zoonosis in addition to armadillos,

more research is needed to determine the exact role of red squirrels in leprosy transmission. Currently, squirrels are not being used as a research model for leprosy.

OTHER RODENTS

Since the earliest days of leprosy research, investigators have tested the utility of different rodent species including hamsters, gerbils, and mystromys. Syrian golden hamsters were inoculated in footpads, ears, and testicles (1, 11, 12). The lesions that developed in the footpads were smaller than those seen in mice. Chinese hamsters and Mongolian gerbils were also tested with similar results to the Syrian golden hamsters. Binford (13) reported successful nerve invasion in inoculated African white-tailed rats (*Mystromys albacaudatus*). However, while these other rodent species have been investigated, the mouse model is still the most commonly used species, and this review will mainly focus on the contributions of the murine model to leprosy research.

In Vivo Infection Models

FOOTPAD INFECTION

Shepard chose the footpad as the site of infection because of its cooler temperature, and because other mycobacteria had been successfully cultured at this site. He found that when approximately 10^4 *M. leprae* were inoculated into the footpad of immunocompetent animals, the bacilli multiplied with a doubling time of ~13 days. Localized growth in the footpad continued until reaching a peak at ~10^6 organisms, at which point the bacilli were killed by the host immune system (14) and slowly cleared. If inoculated with ≥10^6 *M. leprae*, there was no bacterial growth as immunization of the mouse occurred at this dose of bacteria. A similar growth plateau of 10^5 to 10^6 organisms is also obtained upon inoculation of rat footpads (15).

Inoculation of *M. leprae* into the footpads of conventional, immunocompetent mice induces a cellular infiltration that is composed mainly of macrophages and epithelioid cells. As the infection progresses, vacuolation increases, bacterial replication ceases, and macrophage activation occurs (16). Numerous lymphocytes are present, but neutrophils are rare. Tissue necrosis does not occur. At this stage of infection, the nerves are not infected. However, neural damage has been reported very late (>2 years) in the course of the infection (17). Bacilli are primarily intracellular, often in clumps, and become progressively more beaded as the infection proceeds past the peak of bacterial growth (see Chapter 5.3).

While Shepard's original model used footpads from immunocompetent mice, other researchers adapted these techniques to immunosuppressed strains to mimic human lepromatous leprosy (LL). The infection of thymectomized and irradiated mice (18, 19), congenitally athymic mice (20,

21), SCID mice (22, 23, 24), neonatally thymectomized rats (25), and athymic rats (26) highlighted the importance of T cells in host defense against leprosy (see Chapter 6.2). As infection progresses over several months, there is a large influx of macrophages into the footpad providing ample cellular substrate for the multiplying bacilli. Eventually, a huge "leproma" consisting of heavily infected foamy macrophages forms and replaces most of the normal footpad tissue (see Chapter 10.2) (21). These macrophages contain hundreds of bacilli with little to no apparent pathological effect on the host, emphasizing the non-toxic nature of this organism (27). Bacterial dissemination is rather slow, but bacilli are eventually found in striated muscle cells, perineural cells, Schwann cells, and fibroblasts. A year after infection, bacteria can be found in virtually all organs except for the central nervous system. Compared to immunocompetent rodents, multiplication of *M. leprae* in immunosuppressed strains often reaches 10^{10} or more bacilli, and such animals have proven useful for the detection of a low proportion of viable *M. leprae* in biopsies from patients following drug treatment (28, 29). Athymic nude mice are now routinely used for cultures of large numbers of viable *M. leprae* for experimental use (30).

While extremely useful, these models did not provide representatives for the investigation of the borderline forms of leprosy (see Chapter 2.4), which are of great clinical importance. The use of gene transfer technology has led to the generation of hundreds of different commercially available genetically engineered mouse strains, such as targeted gene knockouts (KO), conditional KO, tissue-specific KO, and knock-in mutations. Other recent approaches include collaborative cross, diversity outbred, and humanized mice (31, 32, 33). The development of these mouse strains has provided new models for investigating infectious diseases.

Leprosy outcomes manifest differently due to the quality of the host immune response (see Chapter 6.1; Chapter 6.2); therefore, mice with defined defects, especially in host defense pathways such as cytokines, chemokines, cytokine and chemokine receptors, immune modulators, and cell surface markers, have been particularly helpful in the study of leprosy pathogenesis. The host cell-mediated immune response to *M. leprae* infection has been evaluated *in vivo* and *in vitro* in multiple KO strains of mice (34, 35, 36, 37, 38, 39). Interestingly, no single KO of a cytokine, T-cell type, or antimicrobial mechanism transformed any of these strains into an immunosuppressed model comparable to the athymic nude mouse. However, based on their unique characteristic profiles, most KO strains could be placed along the leprosy spectrum (see Chapter 2.4). These findings may help to elucidate mechanisms that may be occurring in borderline leprosy.

INTRA-NERVE INFECTION

M. leprae and *M. lepromatosis* are the only bacteria that naturally infect human nerves, having a tropism for Schwann cells. However, *M. leprae* do not readily infect murine Schwann cells *in vivo*, presumably due to a lack of appropriate receptors. Shetty et al. (40, 41, 42, 43) inoculated $1–2x10^7$ viable *M. leprae* directly into the sciatic nerves of immunocompetent and immunosuppressed mice. Although *M. leprae* were not observed in the Schwann cells of either strain, a tuberculoid-type granulomatous response (see Chapter 2.4) was elicited in the immunocompetent

mice, whereas immunosuppressed mice developed a macrophage response. Interestingly, both bacterial growth and bacterial viability were decreased after intra-neural inoculation, possibly due to the warm temperature of the sciatic nerve. Rambukkana et al. (44) reported substantial demyelination in both immunocompetent and *Rag1-/-* mice, which lack T and B cells, after intra-neural inoculation of live *M. leprae,* dead *M. leprae*, or whole cell wall fraction. Thus, while murine intra-neural inoculation may not be representative of a natural infection with *M. leprae*, these studies show that this route of administration is an effective method for investigating both immunologically and non-immunologically mediated nerve damage in leprosy.

EAR INFECTION

Studies of cutaneous leishmaniasis utilized a murine ear pinnae model and discovered interesting differences in disease development and anti-Leishmania immune responses in the ear pinnae infection versus the footpad infection (45). Since murine ear pinnae are also cooler areas similar to footpads, intradermal inoculation of viable *M. leprae* into ear pinnae was attempted to study the growth kinetics and development of anti-*M. leprae* immune responses (46). By 30 weeks post inoculation, there was limited growth of *M. leprae* in the ears, and the draining lymph nodes showed markedly more cellular infiltration, especially with regard to IFNγ-secreting CD4+ T cells, compared to that of footpad-inoculated mice. The cellular infiltration in draining lymph nodes as well as at the inoculation site was significantly reduced following rifampin treatment of mice shortly (1–3 weeks) after inoculation (see Chapter 5.2). This modulatory inflammatory response seen in ear pinnae was proposed as a potential model for testing new anti-leprosy drugs.

In Vitro Infection Models

MACROPHAGES

In 1965, Chang and Neikirk presented one of the earliest reports of the use of murine macrophages for leprosy research at the joint conference of The Leonard Wood Memorial and The Armed Forces Institute of Pathology (47). Mouse peritoneal macrophages were infected *in vitro* with *M. leprae* obtained from 11 different patient biopsies from varied geographical regions. The infected cultures were maintained over 10 weeks, and the authors reported elongation of the intracellular AFB. A much later study, using nude MFP-derived *M. leprae* inoculated into mouse peritoneal macrophages, also showed bacterial elongation but no growth (48). Moreover, this latter study established that IL-10 is needed to maintain intracellular bacterial metabolic activity, as measured by palmitic acid oxidation rate (see Chapter 5.3). Without IL-10, metabolic activity of intracellular bacteria maintained within macrophages *in vitro* significantly declined within 3–4 weeks.

Krahenbuhl and his group were instrumental in establishing the use of *in vitro* murine macrophage cultures as a valuable model for understanding basic leprosy immunology. Using this macrophage model, his group established that infected mouse peritoneal macrophages become refractory to IFN-γ induced macrophage activation both *in vivo* and *in vitro* (49, 50). Further research found the fate of live versus dead *M. leprae* in macrophages to be quite different. Live *M. leprae* prevented phagolysosomal fusion whereas phagolysosomal fusion occurred in macrophages when infected with gamma-irradiated (dead) *M. leprae* (51). This finding was one of the first indications that live and dead bacilli are perceived differently by the host cell.

Using fluorescent microscopy, Alves et al. (52) examined the colocalization of viable or heat-killed *M. leprae* bacilli within the acidified lysosomal compartments in RAW (mouse) macrophages and observed differences in endosomal trafficking of live versus dead *M. leprae* in the cell line. The viability of intracellular bacilli rapidly decreases after macrophage activation, and this intracellular killing is dependent on the L-arginine-dependent production of reactive nitrogen intermediates (53, 54). Together, these data suggest that following infection, viable *M. leprae* creates a niche for itself by resisting lysosomal digestion and inhibiting IFN-γ induced activation, as macrophages become potent killers of intracellular bacilli following activation.

GRANULOMA CELLS

The MFP model also allowed for the immunological investigation of the *M. leprae*-induced granuloma (see Chapter 2.4). After infection with *M. leprae*, T cells and macrophages are the primary cells that respond to the infection. However, because of leprosy's extremely slow progression and its capacity to remain asymptomatic long term, it is difficult to define the early cellular interactions of this immune response to infection. Therefore, neither the dynamics of the T-cell and macrophage interactions within the microenvironment of the granulomatous lesion nor their role in disease development and progression are fully understood.

Granuloma cells can be isolated from the footpad and cultured *in vitro* (27, 49). Studies using such cells showed that heavily infected granuloma macrophages are refractory to IFN-γ activation (49, 55) and that this unresponsiveness may be due to elevated production of prostaglandin E2 (50, 56). Observations of inflammation in the footpads has been correlated with distinct immune cell phenotypes and cytokine and chemokine expression and production of the granuloma cells (39).

SCHWANN CELLS

M. leprae and *M. lepromatosis* are the only bacteria known to invade peripheral nerves in humans, where they show a unique tropism towards Schwann cells (see Chapter 9.1; Chapter 9.2). Due to the inherent difficulties in obtaining human peripheral nerve tissues, much of our understanding of *M. leprae*'s interactions with peripheral nerves comes from experiments with mouse or rat primary Schwann cells. These rodent studies have contributed significantly to our understanding of *M. leprae* pathogenesis in peripheral nerves.

An early study in the 1980s found that Schwann cells (primary rat sciatic nerve derived) lacked an *M. leprae*-specific uptake system because *M. leprae*, along with other mycobacterium species and inert particles (latex beads), were all phagocytosed indiscriminately (57). However, another study showed that lipase pretreatment of mouse Schwann cells decreased *M. leprae* adherence, while trypsin pretreatment enhanced adhesion. Based on these findings, *M. leprae* adhesion to Schwann cells appears dependent on surface lipid receptors (58).

Using primary rat Schwann cells, Rambukkana et al. (58, 59, 60, 61) showed that *M. leprae* specifically binds to the G-domain of the laminin-α2 chain present on Schwann cell basal lamina and utilizes it as a bridge molecule to ultimately interact with laminin receptor β_4-integrin for internalization. PGL-1, a molecule specific to the *M. leprae* cell wall, binds laminin-α2 with its terminal trisaccharide. It appears that entry of *M. leprae* into Schwann cells is a complex process involving at least two phases, binding to basal lamina of the Schwann cells-axon unit and then invading Schwann cells, involving multiple host and bacterial molecules.

Both myelinating and non-myelinating Schwann cells are able to phagocytose *M. leprae* with similar efficiencies. In a rat sciatic nerve Schwann cells-neuron co-culture model, infected Schwann cells can myelinate without any apparent difference in myelin architecture compared to uninfected Schwann cells. However, another study using a rat Schwann cells-dorsal root ganglion neuron co-culture showed rapid demyelination of the myelinated Schwann cells within 24 hours of bacterial invasion without any indications of apoptosis and without any inflammatory cells. This bacterial-induced demyelination is independent of bacterial viability because demyelination also occurs with irradiated (dead) bacilli (44, 62).

Both adult and neonatal rat Schwann cells are able to produce IL-1 in response to *M. leprae* antigens, which allows the Schwann cells to function as antigen-presenting cells (63, 64, 65). Ford and Britton conclusively showed that rat Schwann cells could present *M. leprae hsp70* to antigen specific T cells. The secondary activation of these T cells was inhibited by anti-MHC-II monoclonal antibodies and not by anti-MHC-I antibodies (66). The ability of Schwann cells to present immunodominant antigens to MHC class II-restricted antigen-specific T lymphocytes should be further investigated to understand its role in the activation of immunological reactions frequently associated with nerve damage.

Viable *M. leprae* are able to induce reprogramming of infected murine Schwann cells through the upregulation of numerous genes responsible for embryonic development, transcription, chromatin remodeling, cell signaling, and cell division. Eventually, these *M. leprae*-infected Schwann cells are reprogrammed into progenitor cells with migratory and immunomodulatory properties conducive to bacterial dissemination. Moreover, the reprogrammed cells can also efficiently transfer bacilli to co-cultured fibroblasts within 24 hours (67, 68, 69). This is a novel phenomenon only observed in *in vitro* mouse primary Schwann cells cultures, but further studies are required to establish whether this process is indeed taking place within the peripheral nerves of leprosy-affected individuals.

Clinical Adjuncts

The inability to culture *M. leprae* in an artificial medium not only impedes the understanding of vital molecular and cellular events in the pathogenesis of leprosy, it also prevents researchers from easily and definitively distinguishing between live and dead bacilli (see Chapter 5.3). The capability to determine bacterial viability is essential for testing the efficacy of new drugs and/or new treatment regimens. While there have been advancements in addressing these issues, such as the use of molecular techniques to measure *M. leprae* viability (70), the MFP model remains the gold standard for the study of leprosy, particularly for the testing of new drugs. New and powerful assays have shed light on many aspects of drug resistance and the response to drug treatments in clinical trials, but all of them rely on the mouse model to be successful.

DIAGNOSTIC ASSAYS

The development of a reliable immune-based laboratory diagnostic test for early or subclinical leprosy is a top priority as early treatment is essential to prevent nerve damage and disability and to block transmission. However, sensitivity is an issue because subclinical leprosy presents minimum immune stimulation. Furthermore, the unknown duration since exposure and the variability in human immune responses confound results. An important advantage of rodent models of infection is that they eliminate many of these issues, especially the problems of unknown length of exposure to *M. leprae* and of potential cross-reactivity with other common mycobacterial species. Hence, the MFP has been utilized in an effort to develop standardized protocols for immunodiagnosis. One such study involved evaluation of the cell-mediated immune response, gauged in terms of IFN-γ secretion, by splenic lymphocytes from infected mice stimulated *in vitro* with *M. leprae* antigens (71). In an attempt to model subclinical or early leprosy, various doses of *M. leprae* and durations of infection were titrated in MFP to establish the minimum dose at the earliest time point required for the development of a measurable cell-mediated immune response. For a consistent and significant response three months after inoculation, a minimum dose of $1x10^5$ live *M. leprae* per footpad was required. Both BALB/c and C57Bl/6 mice responded similarly although the response was more robust in C57Bl/6 mice.

This model was able to pick-up most, though not all, of the recombinant *M. leprae* antigens recognized by patient sera or peripheral blood mononuclear cells (71, 72, 73). Thus, this 'early' disease model may be used to screen *M. leprae* antigens for immunodiagnostic purposes that may then be further evaluated in the natural hosts of *M. leprae*. T cells isolated from the site of an *M. leprae* infection (i.e., *M. leprae*-induced foot pad granuloma) could also be examined for their antigen responsiveness (36, 37).

DRUG TRIALS AND DRUG RESISTANCE

Since being introduced in 1981 by the World Health Organization, multi-drug therapy (MDT; see Chapter 2.6) has been used successfully to treat leprosy. However, the number of new leprosy cases each year has not shown a significant decrease and the presence of drug resistance remains a concern, indicating a need for new drugs to treat leprosy.

The mouse model has been used extensively for testing new drug therapies for leprosy (74). The ability to test new antibiotics in the MFP is part of a very useful and successful drug pipeline (Figure 1). The pipeline consists of three stages: (1) determining efficacy against non-dividing *M. leprae* in axenic cultures, (2) determining efficacy against non-dividing intracellular *M. leprae* in macrophage cultures, and (3) determining efficacy against multiplying *M. leprae* in MFP assays. The MFP step is traditionally performed using immunocompetent mouse strains, such as Balb/c or Swiss Webster, to test the proposed drug for bactericidal and bacteriostatic effects against multiplying bacteria (75, 76, 77, 78). However, because this assay has limited sensitivity, it can be

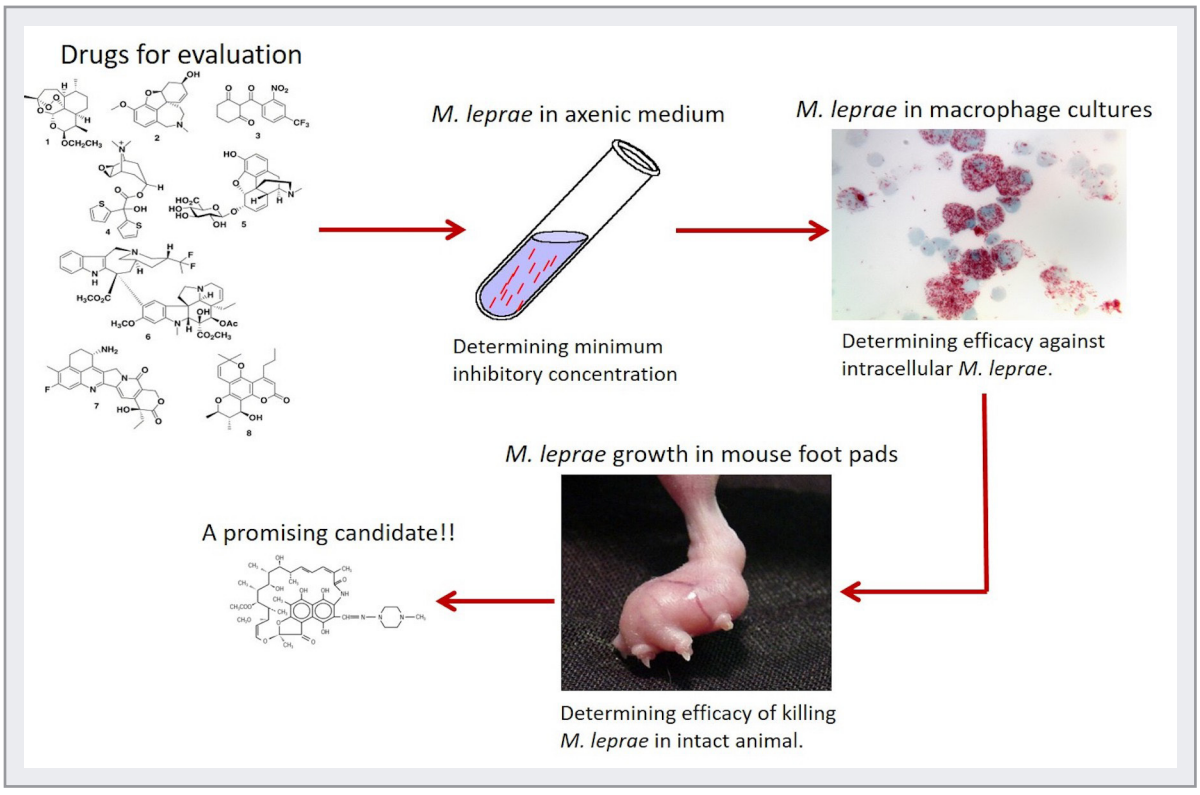

FIG 1 NHDP drug screening pipeline.

Test drugs, which have been verified to have a target in *M. leprae*, are screened for bactericidal activity against non-dividing bacilli in axenic culture and non-dividing intracellular bacilli in macrophage culture. Drugs showing activity are then evaluated for both bactericidal and bacteriostatic effects against multiplying *M. leprae* in the MFP assay.

modified to include athymic nude mice for a more robust measure of drug efficacy. This model has been used to test the efficacy of several different compounds, including PA-824, nitazoxanide, and rifapentine (79, 80).

While these studies have shown promise for new therapies, potential resistance to current drug regimens remains an issue. For years, the mouse model was the only reliable and accurate method to test for drug resistance in *M. leprae*. However, the time, costs, and technical expertise associated with using the mouse model, as well as advancements in molecular techniques (81, 82), has led to a decline in the usage of the mouse model for detecting drug resistance in most laboratories.

SCREENING VACCINE CANDIDATES

In addition to their usefulness in drug studies, murine models are beneficial for screening potential leprosy vaccine candidates (see Chapter 6.3). The concept of utilizing a vaccine that is cross protective for both tuberculosis and leprosy, two poverty-associated diseases caused by closely related organisms, has been considered for years. The most widely used and studied vaccine is live attenuated *M. bovis* Bacille Calmette-Guérin (BCG), which currently remains the only global TB vaccine (83), and numerous studies have shown it has some effectiveness against leprosy as well (see Chapter 6.3). An early study by Shepard et al. (84) used Swiss Webster mice to measure the immune response elicited by vaccines of either *M. bovis* BCG, heat-killed *M. leprae*, or a combination. In terms of sensitization, all three produced similar immune responses except at the lowest dose, where the combination was more effective. In human trials, BCG has variable protection against leprosy, ranging from an average of 26% in experimental studies to an average of 61% in observational studies (85). A vaccine composed of *M. indicus pranii* has also shown protection against both diseases (86) and is being tested as an adjunct to chemotherapy (87, 88).

Recombinant protein vaccines are another option. LepVax (89) is composed of three different antigens (ML2055, ML2380, and ML2028) that have been shown to provoke a robust immune response (see Chapter 6.3). A Th1-stimulating adjuvant, GLA-SE, is added to the vaccine to elicit a stronger cell-mediated immune response. When C57BL/6 mice were immunized with LepVax and challenged with *M. leprae*, the bacterial load in the immunized mice was 85% less compared to mice immunized with GLA-SE alone. Another study comparing *M. tuberculosis*-Ag85B-ESAT6/GLA-SE with *M. leprae* Ag85B-ESAT6/GLA-SE (90) showed that the *M. tuberculosis* formulation protected against *M. leprae* infection significantly better than the *M. leprae* formulation, providing optimism for a successful cross-protective vaccine.

RELAPSE VERSUS REINFECTION

Currently, there is no clinical methodology for distinguishing between relapse and reinfection in leprosy cases. Relapse is a critical indicator of drug therapy effectiveness, while reinfection is

more indicative of active transmission. As there is no way of discerning between the two, both researchers and clinicians struggle to ascertain the effectiveness of a given drug therapy. A few studies in recent years have demonstrated the ability to distinguish between relapse and reinfection by using molecular genotyping techniques, including typing specific single nucleotide polymorphisms (SNPs) and variable number tandem repeats (VNTR) in *M. leprae* (91, 92, 93, 94, 95). While these approaches have been shown to be effective, they still face limitations in addressing this issue.

A recent study by Stefani et al. (96) showed that a whole-genome sequencing analysis of *M. leprae* from recurrent leprosy patients can characterize the recurrences as relapse, reinfection, or drug resistance. Samples from two of the three patients in the study were passaged in BALB/c mice, and then DNA was extracted and sheared to prepare a library for whole genome sequencing. Overall, they were able to show that two of the three cases of recurrence were due to relapse, while the third was due to reinfection. More studies are needed to adequately address if recurrent cases of leprosy are due to relapse or reinfection, and mice continue to serve as an valuable resource in this endeavor.

LIMITATIONS OF RODENT MODELS

Although rodents are a vital resource for research, investigators must be cognizant of the immunological differences between rodents and humans. Immunocompetent mice are highly resistant to infection with *M. leprae*, and they are not an ideal model for the study of nerve infection with the bacilli. They also lack a homologue for granulysin, a key anti-mycobacterial protein generated by human NK and CD8+ T cells (97) (see Chapter 6.2). In addition, NOS2-generated reactive nitrogen intermediates, which are toxic radicals of activated macrophages that have antimycobacterial properties (53, 98), are produced much more robustly by murine macrophages compared to human macrophages (99, 100). Mouse strains also vary in their ability to recognize certain mycobacterial antigens such as CFP-10 (71, 101). Another drawback to the mouse model is its inability to display the full clinical manifestations of the leprosy spectrum that is seen in humans. Fortunately, genetic knockout strains of mice can address this issue in many cases (38). Finally, there is no known rodent model, or any animal model for that matter, for leprosy reactions, which is a critical shortcoming for the investigation of these serious phenomena.

Despite these drawbacks, rodents, especially mice, are invaluable models for leprosy studies. With the ease of use, availability of numerous biological reagents, availability of numerous genetically defined strains, and readily assessable granulomatous footpad lesions, mice are ideal for studying the basic immunological parameters of infection. For many years they have also proven useful for the evaluation of potential new anti-leprosy drugs and to determine experimental vaccine efficacy, which further confirms their status as the gold standard animal model for leprosy research.

References

1. **Shepard CC.** 1960. The experimental disease that follows the injection of human leprosy bacilli into foot-pads of mice. J Exp Med **112**:445–454.

2. **Johnstone PA.** 1987. The search for animal models of leprosy. Int J Lepr Other Mycobact Dis **55**(3):535–547.

3. **Shepard CC.** 1962. Multiplication of *Mycobacterium leprae* in the foot-pad of the mouse. Int J Lepr **30**:291–306.

4. **Rees RJ.** 1964. Limited multiplication of acid-fast bacilli in the foot-pads of mice inoculated with *Mycobacterium leprae*. Brit J Exp Pathol **45**:207–218.

5. **Pattyn SR.** 1965. Comparative behaviour of a strain of *M. leprae* in 5 different mouse strains and in thymectomized mice. Zentralbl Bakteriol Orig **197**(2):256–258.

6. **Kuramoto T, Nakanishi S, Ochiai M, Nakagama H, Voigt B, Serikawa T.** 2012. Origins of albino and hooded rats: implications from molecular genetic analysis across modern laboratory rat strains. PLoS One **7**(8):e43059.

7. **Gibbs RA, Weinstock GM, Metzker ML, Muzny DM, Sodergren EJ, Scherer S, et al.** 2004. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. Nature **428**(6982):493–521.

8. **Pritchett KR, Corning BF.** 2004. Biology and medicine of rats. *In* Reuter JD, Suckow MA (eds), Laboratory animal medicine and management. Ithaca, NY: International Veterinary Information Service.

9. **Avanzi C, Del-Pozo J, Benjak A, Stevenson K, Simpson VR, Busso P, McLuckie J, Loiseau C, Lawton C, Schoening J, Shaw DJ, Piton J, Vera-Cabrera L, Velarde-Felix JS, McDermott F, Gordon SV, Cole ST, Meredith AL.** 2016. Red squirrels in the British Isles are infected with leprosy bacilli. Science **354**(6313):744–747.

10. **Schilling AK, Avanzi C, Ulrich RG, Busso P, Pisanu B, Ferrari N, Romeo C, Mazzamuto MV, McLuckie J, Shuttleworth CM, Del-Pozo J, Lurz PWW, Escalante-Fuentes WG, Ocampo-Candiani J, Vera-Cabrera L, Stevenson K, Chapuis JL, Meredith AL, Cole ST.** 2019. British red squirrels remain the only known wild rodent host for leprosy bacilli. Front Vet Science **6**:8.

11. **Binford CH.** 1965. Transmission of *Mycobacterium leprae* to animals. Nerve involvement in the ears of hamsters. Int J Lepr **33**(4):865–874.

12. **Waters MF, Niven JS.** 1965. Experimental infection of the golden hamster with *Mycobacterium leprae*. Int J Lepr **33**(3):297–315.

13. **Binford CH.** 1965. The transmission of M. leprae to animals. Attempts to find an experimental model. Int J Lepr **36**(4):599.

14. **Welch TM, Gelber RH, Murray LP, Ng H, O'Neill SM, Levy L.** 1980. Viability of *Mycobacterium leprae* after multiplication in mice. Infect Immun **30**(2):325–328.

15. **Hilson GR.** 1965. Observations on the inoculation of *M. leprae* in the foot pad of the white rat. Int J Lepr **33**(3):Suppl:662–666.

16. **Evans MJ, Levy L.** 1972. Ultrastructural changes in cells of the mouse footpad infected with *Mycobacterium leprae*. Infect Immun **5**(2):238–247.

17. **Rees RJ, Weddell AG, Palmer E, Pearson JM.** 1969. Human leprosy in normal mice. Brit Med J **3**(5664):216–217.

18. **Rees RJ.** 1966. Enhanced susceptibility of thymectomized and irradiated mice to infection with *Mycobacterium leprae*. Nature **211**(5049):657–658.

19. **Ebenezer GJ, Arumugam S, Job CK.** 2002. Dosage and site of entry influence growth and dissemination of *Mycobacterium leprae* in T900r mice. Int J Lepr Other Mycobact Dis **70**(4):245–249.

20. **Colston MJ, Hilson GR.** 1976. Growth of *Mycobacterium leprae* and *M. marinum* in congenitally athymic (nude) mice. Nature **262**(5567):399–401.

21. **Chehl S, Ruby J, Job CK, Hastings RC.** 1983. The growth of *Mycobacterium leprae* in nude mice. Lepr Rev **54**(4):283–304.

22. **Yogi Y, Nakamura K, Inoue T, Kawatsu K, Kashiwabara Y, Sakamoto Y, Izumi S, Saito M, Hioki K, Nomura T.** 1991. Susceptibility of severe combined immunodeficient (SCID) mice to *Mycobacterium leprae*: multiplication of the bacillus and dissemination of the infection at early stage. Nihon Rai Gakkai Zasshi **60**(3–4):139–145.

23. **Azouaou N, Gelber RH, Abel K, Sasaki DT, Murray LP, Locksley RM, Mohagheghpour N.** 1993. Reconstitution of *Mycobacterium leprae* immunity in severe combined immunodeficient mice using a T-cell line. Int J Lepr Other Mycobact Dis **61**(3):398–405.

24. **Ishaque M, Sticht-Groh V.** 1994. Experimental transmission of human leprosy bacilli in foot pads of severe combined immunodeficient mice. Int J Lepr Other Mycobact Dis **62**(4):613–614.

25. **Gelber RH.** 1987. The neonatally thymectomized rat as a model of the lepromatous patient. Int J Lepr Other Mycobact Dis **55**(4 Suppl):879–881.

26. **Dawson PJ, Colston MJ, Fieldsteel AH.** 1983. Infection of the congenitally athymic rat with *Mycobacterium leprae*. Int J Lepr Other Mycobact Dis **51**(3):336–346.

27. **Hagge DA, Ray NA, Krahenbuhl JL, Adams LB.** 2004. An in vitro model for the lepromatous leprosy granuloma: fate of *Mycobacterium leprae* from target macrophages after interaction with normal and activated effector macrophages. J Immunol **172**(12):7771–7779.

28. **Colston MJ.** 1987. Application of the thymectomized-irradiated mouse to the detection of persisting *Mycobacterium leprae*. Int J Lepr Other Mycobact Dis **55**(4 Suppl):859–863.

29. **Gelber RH, Levy L.** 1987. Detection of persisting *Mycobacterium leprae* by inoculation of the neonatally thymectomized rat. Int J Lepr Other Mycobact Dis **55**(4 Suppl):872–878.

30. **Truman RW, Krahenbuhl JL.** 2001. Viable *M. leprae* as a research reagent. Int J Lepr Other Mycobact Dis **69**(1):1–12.

31. **Smith CM, Proulx MK, Olive AJ, Laddy D, Mishra BB, Moss C, Gutierrez NM, Bellerose MM, Barreira-Silva P, Phuah JY, Baker RE, Behar SM, Kornfield H, Evans TG, Beamer G, Sassetti CM.** 2016. Tuberculosis susceptibility and vaccine protection are independently controlled by host genotype. mBio **7**(5):e01516-16.

32. **Niazi MKK, Dhulekar N, Schmidt D, Major S, Cooper R, Abeijon C, Gatti DM, Kramnik I, Yener B, Gurcan M, Beamer G.** 2015. Lung necrosis and neutrophils reflect common pathways of susceptibility to *Mycobacterium tuberculosis* in genetically diverse, immune-competent mice. Dis Models Mech **8**(9):1141–1153.

33. **Arrey F, Lowe D, Kuhlmann S, Kaiser P, Moura-Alves P, Krishnamoorthy G, Lozza L, Maertzdorf J, Skrahina T, Skrahina A, Gengenbacher M, Nouailles G, Kaufmann SHE.** 2019. Humanized mouse model mimicking pathology of human tuberculosis for *in vivo* evaluation of drug regimens. Front Immunol **10**:89.

34. **Adams LB, Job CK, Krahenbuhl JL.** 2000. Role of inducible nitric oxide synthase in resistance to *Mycobacterium leprae* in mice. Infect Immun **68**(9):5462–5465.

35. **Adams LB, Scollard DM, Ray NA, Cooper AM, Frank AA, Orme IM, Krahenbuhl JL.** 2002. The study of *Mycobacterium leprae* infection in interferon-gamma gene—disrupted mice as a model to explore the immunopathologic spectrum of leprosy. J Infect Dis **185 Suppl 1**:S1–S8.

36. **Hagge DA, Marks VT, Ray NA, Dietrich MA, Kearney MT, Scollard DM, Krahenbuhl JL, Adams LB.** 2007. Emergence of an effective adaptive cell mediated immune response

to *Mycobacterium leprae* is not impaired in reactive oxygen intermediate-deficient mice. FEMS Immunol Med Microbiol **51**(1):92–101.

37. **Hagge DA, Saunders BM, Ebenezer GJ, Ray NA, Marks VT, Britton WJ, Krahenbuhl JL, Adams LB.** 2009. Lymphotoxin-alpha and TNF have essential but independent roles in the evolution of the granulomatous response in experimental leprosy. Am J Pathol **174**(4):1379–1389.

38. **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 Instit Oswaldo Cruz **107 Suppl 1**:197–208.

39. **Hagge DA, Scollard DM, Ray NA, Marks VT, Deming AT, Spencer JS, Adams LB.** 2014. IL-10 and NOS2 modulate antigen-specific reactivity and nerve infiltration by T cells in experimental leprosy. PLoS Negl Trop Dis **8**(9):e3149.

40. **Shetty VP.** 1993. Animal model to study the mechanism of nerve damage in leprosy—a preliminary report. Int J Lepr Other Mycobact Dis **61**(1):70–75.

41. **Birdi TJ, Shetty VP, Antia NH.** 1995. Differences in *M. leprae*-induced nerve damage in Swiss white and C57BL/6 mice. Int J Lepr Other Mycobact Dis **63**(4):573–574.

42. **Shetty VP, Mistry NF, Birdi TJ, Antia NH.** 1995. Effect of T-cell depletion on bacterial multiplication and pattern of nerve damage in *M. leprae*-infected mice. Indian J Lepr **67**(4):363–374.

43. **Shetty VP, Matharu PS, Antia NH.** 1999. Sciatic nerve of normal and T200x5R Swiss white mice fails to support multiplication of intraneurally injected *M. leprae*. Int J Lepr Other Mycobact Dis **67**(4):446–452.

44. **Rambukkana A, Zanazzi G, Tapinos N, Salzer JL.** 2002. Contact-dependent demyelination by *Mycobacterium leprae* in the absence of immune cells. Science **296**(5569):927–931.

45. **Baldwin TM, Elso C, Curtis J, Buckingham L, Handman E.** 2003. The site of *Leishmania major* infection determines disease severity and immune responses. Infect Immun **71**(12):6830–6834.

46. **Duthie MS, Reece ST, Lahiri R, Goto W, Raman VS, Kaplan J, Ireton GC, Bertholet S, Gillis TP, Krahenbuhl JL, Reed SG.** 2007. Antigen-specific cellular and humoral responses are induced by intradermal *Mycobacterium leprae* infection of the mouse ear. Infect Immun **75**(11):5290–5297.

47. **Chang YT, Neikirk RL.** 1965. *Mycobacterium lepraemurium* and *Mycobacterium leprae* in cultures of mouse peritoneal macrophages (preliminary results). Int J Lepr **33**(3):Suppl:586–603.

48. **Fukutomi Y, Matsuoka M, Minagawa F, Toratani S, McCormick G, Krahenbuhl J.** 2004. IL-10 treatment of macrophages bolsters intracellular survival of *Mycobacterium leprae*. Int J Lepr Other Mycobact Dis **72**(1):16–26.

49. **Sibley LD, Krahenbuhl JL.** 1987. *Mycobacterium leprae*-burdened macrophages are refractory to activation by gamma interferon. Infect Immun **55**(2):446–450.

50. **Sibley LD, Krahenbuhl JL.** 1988. Induction of unresponsiveness to gamma interferon in macrophages infected with *Mycobacterium leprae*. Infect Immun **56**(8):1912–1919.

51. **Sibley LD, Franzblau SG, Krahenbuhl JL.** 1987. Intracellular fate of *Mycobacterium leprae* in normal and activated mouse macrophages. Infect Immun **55**(3):680–685.

52. **Alves L, de Mendonca Lima L, da Silva Maeda E, Carvalho L, Holy J, Sarno EN, Pessolani MCV, Barker LP.** 2004. *Mycobacterium leprae* infection of human Schwann cells depends on selective host kinases and pathogen-modulated endocytic pathways. FEMS Microbiol Lett **238**(2):429–437.

53. **Adams LB, Franzblau SG, Vavrin Z, Hibbs JB Jr, Krahenbuhl JL.** 1991. L-arginine-dependent macrophage effector functions inhibit metabolic activity of *Mycobacterium leprae*. J Immunol **147**(5):1642–1646.

54. **Ramasesh N, Adams LB, Franzblau SG, Krahenbuhl JL.** 1991. Effects of activated macrophages on *Mycobacterium leprae*. Infect Immun **59**(9):2864–2869.

55. **Sibley LD, Krahenbuhl JL.** 1988. Defective activation of granuloma macrophages from *Mycobacterium leprae*-infected nude mice. J Leukoc Biol **43**(1):60–66.

56. **Adams LB, Gillis TP, Hwang DH, Krahenbuhl JL.** 1997. Effects of essential fatty acid deficiency on prostaglandin E2 production and cell-mediated immunity in a mouse model of leprosy. Infect Immun **65**(4):1152–1157.

57. **Band AH, Bhattacharya A, Talwar GP.** 1986. Lack of *Mycobacterium leprae*-specific uptake in Schwann cells. Int J Lepr Other Mycobact Dis **54**(1):71–78.

58. **Rambukkana A, Salzer JL, Yurchenco PD, Tuomanen EI.** 1997. Neural targeting of *Mycobacterium leprae* mediated by the G domain of the laminin-alpha2 chain. Cell **88**(6):811–821.

59. **Rambukkana A, Yamada H, Zanazzi G, Mathus T, Salzer JL, Yurchenco PD, Campbell KP, Fischetti VA.** 1998. Role of alpha-dystroglycan as a Schwann cell receptor for *Mycobacterium leprae*. Science **282**(5396):2076–2079.

60. **Shimoji Y, Ng V, Matsumura K, Fischetti VA, Rambukkana A.** 1999. A 21-kDa surface protein of *Mycobacterium leprae* binds peripheral nerve laminin-2 and mediates Schwann cell invasion. Proc Nat Acad Sci USA **96**(17):9857–9862.

61. **Ng V, Zanazzi G, Timpl R, Talts JF, Salzer JL, Brennan PJ, Rambukkana A.** 2000. Role of the cell wall phenolic glycolipid-1 in the peripheral nerve predilection of *Mycobacterium leprae*. Cell **103**(3):511–524.

62. **Hagge DA, Oby Robinson S, Scollard D, McCormick G, Williams DL.** 2002. A new model for studying the effects of *Mycobacterium leprae* on Schwann cell and neuron interactions. J Infect Dis **186**(9):1283–1296.

63. **Samuel NM, Jessen KR, Grange JM, Mirsky R.** 1987. Gamma interferon, but not *Mycobacterium leprae*, induces major histocompatibility class II antigens on cultured rat Schwann cells. J Neurocytol **16**(2):281–287.

64. **Kingston AE, Bergsteinsdottir K, Jessen KR, Van der Meide PH, Colston MJ, Mirsky R.** 1989. Schwann cells co-cultured with stimulated T cells and antigen express major histocompatibility complex (MHC) class II determinants without interferon-gamma pretreatment: synergistic effects of interferon-gamma and tumor necrosis factor on MHC class II induction. Eur J Immunol **19**(1):177–183.

65. **Bergsteinsdottir K, Kingston A, Mirsky R, Jessen KR.** 1991. Rat Schwann cells produce interleukin-1. J Neuroimmunol **34**(1):15–23.

66. **Ford AL, Britton WJ, Armati PJ.** 1993. Schwann cells are able to present exogenous mycobacterial hsp70 to antigen-specific T lymphocytes. J Neuroimmunol **43**(1–2):151–159.

67. **Masaki T, McGlinchey A, Tomlinson SR, Qu J, Rambukkana A.** 2013. Reprogramming diminishes retention of *Mycobacterium leprae* in Schwann cells and elevates bacterial transfer property to fibroblasts. F1000Res **2**:198.

68. **Masaki T, Qu J, Cholewa-Waclaw J, Burr K, Raaum R, Rambukkana A.** 2013. Reprogramming adult Schwann cells to stem cell-like cells by leprosy bacilli promotes dissemination of infection. Cell **152**(1–2):51–67.

69. **Masaki T, McGlinchey A, Cholewa-Waclaw J, Qu J, Tomlinson SR, Rambukkana A.** 2014. Innate immune response precedes *Mycobacterium leprae*-induced reprogramming of adult Schwann cells. Cell Reprogram **16**(1):9–17.

70. **Davis GL, Ray NA, Lahiri R, Gillis TP, Krahenbuhl JL, Williams DL, Adams LB.** 2013. Molecular assays for determining *Mycobacterium leprae* viability in tissues of experimentally infected mice. PLoS Negl Trop Dis **7**(8):e2404.

71. **Lahiri R, Randhawa B, Franken KL, Duthie MS, Spencer JS, Geluk A, Krahenbuhl JL.** 2011. Development of a mouse food pad model for detection of sub clinical leprosy. Lepr Rev **82**(4):432–444.

72. **Spencer JS, Kim HJ, Wheat WH, Chatterjee D, Balagon MV, Cellona RV, Tan EV, Gelber R, Saunderson P, Duthie MS, Reece ST, Burman W, Belknap R, Mac Kenzie WR, Geluk A, Oskam L, Dockrell HM, Brennan PJ, IDEAL Consortium.** 2011. Analysis of antibody responses to *Mycobacterium leprae* phenolic glycolipid I, lipoarabinomannan, and recombinant proteins to define disease subtype-specific antigenic profiles in leprosy. Clin Vaccine Immunol **18**(2):260–267.

73. **Sampaio LH, Stefani MM, Oliveira RM, Sousa AL, Ireton GC, Reed SG, Duthie MS.** 2011. Immunologically reactive *M. leprae* antigens with relevance to diagnosis and vaccine development. BMC Infect Dis **11**:26.

74. **Levy L, Ji B.** 2006. The mouse foot-pad technique for cultivation of *Mycobacterium leprae*. Lepr Rev **77**(1):5–24.

75. **McDermott-Lancaster RD, Banerjee DK.** 1993. Activity of sparfloxacin against *Mycobacterium leprae* measured by the proportional bactericidal test. Int J Lepr Other Mycobact Dis **61**(4):605–608.

76. **Ji B, Grosset J.** 2000. Combination of rifapentine-moxifloxacin-minocycline (PMM) for the treatment of leprosy. Lepr Rev **71 Suppl**:S81–S87.

77. **Ji B, Chauffour A, Andries K, Jarlier V.** 2006. Bactericidal activities of R207910 and other newer antimicrobial agents against *Mycobacterium leprae* in mice. Antimicrob Agents Chemother **50**(4):1558–1560.

78. **Randhawa B, Harris EB, Prabhakaran K.** 1999. Bactericidal action of oral ampicillin/sulbactam against *Mycobacterium leprae*. J Antimicrob Chemother **44**(2):279–281.

79. **Manjunatha UH, Lahiri R, Randhawa B, Dowd CS, Krahenbuhl JL, Barry CE 3rd.** 2006. *Mycobacterium leprae* is naturally resistant to PA-824. Antimicrob Agents Chemother **50**(10):3350–3354.

80. **Bailey MA, Na H, Duthie MS, Gillis TP, Lahiri R, Parish T.** 2017. Nitazoxanide is active against *Mycobacterium leprae*. PLoS One **12**(8):e0184107.

81. **Araujo S, Goulart LR, Truman RW, Goulart IMB, Vissa V, Li W, Matsuoka M, Suffys P, Fontes AB, Rosa PS, Scollard DM, Williams DL.** 2017. qPCR-high resolution melt

analysis for drug susceptibility testing of *Mycobacterium leprae* directly from clinical specimens of leprosy patients. PLoS Negl Trop Dis **11**(6):e0005506.

82. **Cambau E, Saunderson P, Matsuoka M, Cole ST, Kai M, Suffys P, Rosa PS, Williams D, Gupta UD, Lavania M, Cardona-Castro N, Miyamoto Y, Hagge D, Srikantam A, Hongseng W, Indropo A, Vissa V, Johnson RC, Cauchoix B, Pannikar VK, Cooreman EAWD, Pemmaraju VRR, Gillini L, WHO surveillance network of antimicrobial resistance in leprosy.** 2018. Antimicrobial resistance in leprosy: results of the first prospective open survey conducted by a WHO surveillance network for the period 2009–15. Clin Microbiol Infect **24**(12):1305–1310.

83. **Luca S, Mihaescu T.** 2013. History of BCG Vaccine. Maedica **8**(1):53–58.

84. **Shepard CC, van Landingham RM, Walker LL, Ye SZ.** 1983. Comparison of the immunogenicity of vaccines prepared from viable *Mycobacterium bovis* BCG, heat-killed *Mycobacterium leprae*, and a mixture of the two for normal and *M. leprae*-tolerant mice. Infect Immun **40**(3):1096–1103.

85. **Setia MS, Steinmaus C, Ho CS, Rutherford GW.** 2006. The role of BCG in prevention of leprosy: a meta-analysis. Lancet Infect Dis **6**(3):162–170.

86. **Singh IG, Mukherjee R, Talwar GP.** 1991. Resistance to intravenous inoculation of *Mycobacterium tuberculosis* H37Rv in mice of different inbred strains following immunization with a leprosy vaccine based on *Mycobacterium w*. Vaccine **9**(1):10–14.

87. **Faujdar J, Gupta P, Natrajan M, Das R, Chauhan DS, Katoch VM, Gupta UD.** 2011. *Mycobacterium indicus pranii* as stand-alone or adjunct immunotherapeutic in treatment of experimental animal tuberculosis. Indian J Med Res **134**(5):696–703.

88. **Kamal R, Natrajan M, Katoch K, Arora M.** 2012. Clinical and histopathological evaluation of the effect of addition of immunotherapy with Mw vaccine to standard chemotherapy in borderline leprosy. Indian J Lepr **84**(4):287–306.

89. **Duthie MS, Pena MT, Ebenezer GJ, Gillis TP, Sharma R, Cunningham K, Polydefkis M, Maeda Y, Makino M, Truman RW, Reed SG.** 2018. LepVax, a defined subunit vaccine that provides effective pre-exposure and post-exposure prophylaxis of *M. leprae* infection. NPJ Vaccines **3**:12.

90. **Coppola M, van den Eeden SJF, Robbins N, Wilson L, Franken KLMC, Adams LB, Gillis TP, Ottenhoff THM, Geluk A.** 2018. Vaccines for leprosy and tuberculosis: opportunities for shared research, development, and application. Front Immunol **9**:308.

91. **Oskam L, Dockrell HM, Brennan PJ, Gillis T, Vissa V, Richardus JH.** 2008. Molecular methods for distinguishing between relapse and reinfection in leprosy. Trop Med Int Health **13**(10):1325–1326.

92. **da Silva Rocha A, Cunha Dos Santos AA, Pignataro P, Nery JA, de Miranda AB, Soares DF, Fontes ANB, Miranda A, Ferreira H, Boéchat N, Gallo MEN, Sarno EN, De Oliveira MLW, Suffys PN.** 2011. Genotyping of *Mycobacterium leprae* from Brazilian leprosy patients suggests the occurrence of reinfection or of bacterial population shift during disease relapse. J Med Microbiol **60**(Pt 10):1441–1446.

93. **Monot M, Honore N, Garnier T, Zidane N, Sherafi D, Paniz-Mondolfi A, Matsuoka M, Taylor GM, Donoghue HD, Bouwman A, Mays S, Watson C, Lockwood D, Khamesipour A, Dowlati Y, Jianping S, Rea TH, Vera-Cabrera L, Stefani MM, Banu S, Macdonald M, Sapkota BR, Spencer JS, Thomas J, Harshman K, Singh P, Busso P, Gattiker A, Rougemont J, Brennan PJ, Cole ST.** 2009. Comparative genomic and phylogeographic analysis of *Mycobacterium leprae*. Nat Genet **41**(12):1282–1289.

94. **Zhang L, Budiawan T, Matsuoka M.** 2005. Diversity of potential short tandem repeats in *Mycobacterium leprae* and application for molecular typing. J Clin Microbiol **43**(10):5221–5229.

95. **Kimura M, Sakamuri RM, Groathouse NA, Rivoire BL, Gingrich D, Krueger-Koplin S, Cho SN, Brennan PJ, Vissa V.** 2009. Rapid variable-number tandem-repeat genotyping for *Mycobacterium leprae* clinical specimens. J Clin Microbiol **47**(6):1757–1766.

96. **Stefani MMA, Avanzi C, Buhrer-Sekula S, Benjak A, Loiseau C, Singh P, Pontes MAA, Gonçalves HS, Hungria EM, Busso P, Piton J, Silveira MIS, Cruz R, Schetinni A, Costa MB, Virmond MCL, Diorio SM, Dias-Baptista IMF, Rosa PS, Matsuoka M, Penna MLF, Cole ST, Penna GO.** 2017. Whole genome sequencing distinguishes between relapse and reinfection in recurrent leprosy cases. PLoS Negl Trop Dis **11**(6):e0005598.

97. **Liu B, Liu S, Qu X, Liu J.** 2006. Construction of a eukaryotic expression system for granulysin and its protective effect in mice infected with *Mycobacterium tuberculosis*. J Med Microbiol **55**(Pt 10):1389–1393.

98. **Chan J, Xing Y, Magliozzo RS, Bloom BR.** 1992. Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. J Exp Med **175**(4):1111–1122.

99. **Weinberg JB, Misukonis MA, Shami PJ, Mason SN, Sauls DL, Dittman WA, Wood ER, Smith GK, McDonald B, Bachus KE, et al.** 1995. Human mononuclear phagocyte inducible nitric oxide synthase (iNOS): analysis of iNOS mRNA, iNOS protein, biopterin, and nitric oxide production by blood monocytes and peritoneal macrophages. Blood **86**(3):1184–1195.

100. **Jung JY, Madan-Lala R, Georgieva M, Rengarajan J, Sohaskey CD, Bange FC, Robinson CM.** 2013. The intracellular environment of human macrophages that produce nitric oxide promotes growth of mycobacteria. Infect Immun **81**(9):3198–3209.

101. **Kamath AB, Woodworth J, Xiong X, Taylor C, Weng Y, Behar SM.** 2004. Cytolytic CD8+ T cells recognizing CFP10 are recruited to the lung after *Mycobacterium tuberculosis* infection. J Exp Med **200**(11):1479–1489.