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REVIEW

# Drug testing in mouse models of tuberculosis and nontuberculous mycobacterial infections

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SUMMARY

Mice as a species are susceptible to tuberculosis infection while mouse inbred strains present wide spectrum of susceptibility/resistance to this infection. However, non-tuberculosis Mycobacterial infections usually cannot be modeled in mice of common inbred strains. Introduction of specific properties, such as gene mutations, recombinants, targeted gene knockouts significantly extended the use of mice to mimic human Mycobacterial infections, including non-tuberculosis ones. This review describes the available mouse models of tuberculosis and non-tuberculosis infections and drug therapy in these models. Mouse models of non-tuberculosis infections are significantly less developed than tuberculosis models, hampering the development of therapies.



The relevance of mouse models for studying tuberculosis (TB) is still a subject of constant debate,[1–4] although experimental approaches that have taken into account genetic diversity of the host provided compelling evidence of their validity (reviewed in[5–7]). Regardless, a rapid and common replacement of mouse models for pre-clinical *in vivo* testing of drug candidates against diseases caused by mycobacteria cannot be realistically expected. With this in mind, we feel it is worthwhile to discuss the main features of such models, paying specific attention to the duration of experiments, reliability of readouts and influence of host genetics. Besides TB, we also briefly review *in vivo* models used for testing drug candidates against non-tuberculous (NTB) mycobacteria.

## 1. Mouse models for TB drug testing

Normally, TB infection in immunocompetent mice causes disease with a prolonged chronic phase: most experiments for pathogenesis and immune response evaluation require several months, and those designed to study latent-reactivation TB require about a year for a single experiment. Such a lengthy experiment can only be performed with the most efficient drug candidate(s), for which subsequent progression to clinical trials is strongly anticipated. Fortunately, several studies demonstrated that a short course treatment is sufficient for estimating drug activity.[8–12] These models are often used as a quick preliminary screen of large numbers of drug candidates, with the most effective drugs moving into evaluation in more “natural” chronic TB models.

One short course model relies on *Mycobacterium tuberculosis*-susceptible mice in which the interferon gamma gene (*ifng*) has been disrupted. In this model, infection manifests as an impetuous mycobacterial growth over 18 days then taken under control by effective compounds after the eight-day treatment regimen.[9,10] Given that the estimation of mycobacterial colony forming unit (CFU) counts from organs takes an addition three weeks, the entire evaluation is completed in ~50 days. Another short course treatment model based upon similar ideas was developed by Matsumoto M. et al.[11] This model relies on infection of athymic T-cell-free nude mice on the BALB/c genetic background. These mice also cannot effectively control *M. tuberculosis* infection, and the timing of experiments is similar to that in the *ifng* knockout (GKO) model.

To further shorten experiments, our group developed a model utilizing infection-induced body weight loss as a surrogate marker of progressive TB infection in immunocompetent but genetically TB-susceptible mice.[8,12] In this body weight loss model, *M. tuberculosis* CFU counts in lungs and spleens directly correlated with body weight loss. Mice of the C3H inbred strain are intravenously inoculated with $10^6$ *M. tuberculosis* CFU and treatment is started one week following challenge and continued for 14 days, for a total experimental duration of 21 days. Ten to 12 days following infection, untreated control mice begin to lose weight, eventually losing approximately 25% of their total body weight by the end of week 3.

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Drug efficacy in this screening model can be estimated with a high degree of accuracy by using the slope of body weight loss from 14 to 15 days until that at 20 days. The slope of weight loss in infected placebo-treated mice was a straight line. Mice treated with effective concentrations of drugs (isoniazid (INH), rifampicin (RIF), etc.) or uninfected mice both had a horizontal or slightly increasing slope. Thus, during 24 h between 15 and 16 days after infection infected placebo-treated mice lost 1.02 ± 0.06 g; mice treated with an effective (standard) dose of INH (25 mg/kg) gained 0.17 ± 0.05 g; mice treated with an insufficient dose of moxifloxacin (MXF) (10 mg/kg) lost 0.50 ± 0.11 g.

Another way to measure drug efficacy in short course treatment models is by estimating the minimal effective dose (MED). The MED is defined as the lowest dose that prevents the development of gross lung lesions and splenomegaly,[13,14] and can only be established in such models: regimens that allow bacteria to freely grow for more than 2–3 weeks normally result in development of gross lesions before treatment commences.

A great number of experimental approaches have been introduced for the assessment of drug candidates in more chronic experimental TB models, in which the extent of disease progression is determined by the dynamic CFU counts in organs. Challenge routes for initiating the infection include moderate to high ($10^5$–$10^7$ CFU), intravenous,[15–28] high ($10^6$–$10^7$ CFU) intranasal,[15,17] or low (<100 CFU) aerosol[10,14,29–37] administrations of *M. tuberculosis*. Besides dynamic CFU counts, drug efficacy is commonly estimated by determining the minimal bactericidal dose (MBD), which is defined as the lowest dose that reduces lung CFU counts by 99% compared to the pretreatment level.[14]

The diversity of methods for infecting mice and measuring the efficacy of therapy resulted in a variety of experimental protocols for drug assessment, each with unique positive and negative features. Under the protocol which is thought to most closely mimic chronic human TB, chemotherapy is initiated not earlier than 3 weeks following challenge, once the infection reaches a more or less stable state.[24,25,28,33–35,38] Other protocols suggest that treatment should be started at the acute phase of infection (0, 1 or 10 days post challenge) in order to mimic either the onset of human infection or the rapid development of TB that can occur in immune compromised individuals.[15,16,21,22,38,39] Finally, some protocols employ an intermediate situation in which chemotherapy is introduced at week 2 post challenge when bacteria are still in the active phase of growth but are beginning to experience the pressure of the host immune reactions.[16,18,19,29,40]

Precise evaluation of treatment efficacy may well be confounded by the presence of non-cultivable mycobacteria, which are physically present in infected tissues but do not grow on agar or even in liquid media *in vitro*. These dormant or latent bacteria can lead to relapse in "cured" mice several months after chemotherapy withdrawal.[41] The probability of a substantially postponed TB relapse makes it difficult to demonstrate that a drug is effective enough to achieve stable sterility that will be maintained through a prolonged post treatment period. In theory, such performance should be considered as the gold standard for drug efficacy testing, because it would suggest that the treatment regimen eradicates both active and dormant bacilli. In practice, the closest approximation to this state was achieved by treating with effective drugs for months, after which lung and spleen homogenates obtained mainly from genetically TB-resistant mice[41] after a 3–6-month resting period provided zero CFU.[21,33–35,40,42]

## 2. Host genetics and anti-TB therapy

It is generally recognized that host genetics has a prominent impact on susceptibility to and severity of mycobacteria-triggered diseases in both mice and humans. This was the subject of several recent reviews.[5,43,44] and will not be discussed here. However, very little is known about the potential influence of host genetics on the performance of anti-TB drugs. In humans, quantitative aspects of treatment were predominantly studied in terms of the interactions of HIV and TB drugs, rather than evaluating the impact of the genetic diversity of patients.[45–48] In mice, no genome-wide screens aimed at the systemic identification of genes that can impact the efficacy of drug treatment have been published to date. Thus, unlike genetic studies in mice of TB infection, which have provided significant data to guide research in humans (see, Ref. [5] for review), researchers have not yet performed the laboratory research that can bridge to studies in humans with respect to the genetically determined differences in drug performance. Nevertheless, we think it is worthwhile to briefly discuss the available experimental results in hopes of drawing attention to this research gap.

Some differences in the efficacy of TB therapy that we can hypothesize may exist could be based in pharmacogenomics, such as variations in drug metabolism or excretion, etc. Pharmacogenomics of TB drugs is determined by the activities of several enzymes, such as *N*-acetyl transferase 2 (NAT2), cytochrome P450 oxidase (CYP2E1) and glutathione *S*-transferase (GSTM1). Polymorphisms at the loci (*NAT2, CYP2E1 and GSTM1*) could modulate the activities of these enzymes and, hence, the risk of hepatotoxicity caused by TB drugs.[49] Works on pharmacogenomics have been made in humans and we do not discussed they here. Differences could also result from disease susceptibility/severity: for example, more susceptible/strongly affected mice could require more intensive therapy. Some interesting data linking TB susceptibility and drug performance in mice have been published.

Significant differences in treatment outcome were shown between immunocompetent and immunocompromised mice. For example, in GKO mice, MXF at a dose of 400 mg/kg for 9 days reduced CFU in lungs from 7 log10 (pretreatment control group) to 2 log10 in treated mice, for a total CFU reduction of 5 logs. The authors[9] suggest that the drug is so efficacious because the very rapid growth of the bacteria in the GKO mice makes them highly susceptible to the drug. In comparison, a comparable level of CFU reduction in immunocompetent BALB/c mice was shown to require treatment for 2 months with MXF in combination with RIF and pyrazinamide (PZA).[35] Contrary, treatment of mice with RIF + INH + PZA was more effective in immunocompetent BALB/c mice than in immune-deficient nude mice.[50] Pharmacokinetic study has shown similar PK indices for BALB/c and nude mice suggesting that the PK is not the cause of the unexpected respond of nude mice to the therapy. Interesting, substitution of RIF for rifapentine (RFP) significantly reduced the differences of drug efficiency for BALB/c and BALB/c-nude mice.

Our recent study compared treatment of C57BL/6, C3H, DBA/2 and SWR/J inbred mouse strains with the combination of four standard drugs: INH, RIF, ethambutol (EMB), and PZA. We demonstrated that in terms of the relative reduction of lung CFU counts compared to untreated control mice, this treatment was the most effective in TB-susceptible DBA/2 and the least of all in TB-resistant C57BL/6 mice.[23] However, a prominent difference in the lung mycobacterial burdens between susceptible and resistant animals at the beginning of treatment made it difficult to draw a clear conclusion.

To more accurately estimate how host genetic susceptibility to infection influences the efficacy of therapy, we modified our model by evaluating different challenge doses in the resistant B6 and susceptible DBA/2 mice. Titration experiments demonstrated that the injection of a 10-fold lower dose in DBA/2 compared to B6 mice resulted in similar lung mycobacterial CFU at week 3 of infection, the time point at which the bacilli switch from exponent to stable