What Animal Models Teach Humans about Tuberculosis

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Animal models have become standard tools for the study of a wide array of human infectious diseases. Although there are no true animal reservoirs for *Mycobacterium tuberculosis*, many different animal species are susceptible to infection with this organism and have served as valuable tools for the study of tuberculosis (TB). The most commonly used experimental animal models of TB are the mouse, rabbit, and guinea pig. Although substantial differences in TB susceptibility and disease manifestations exist between these species, they have contributed significantly to the understanding of TB immunopathogenesis, host genetic influence on infection, efficacy of antimicrobial therapy, and host/pathogen interactions that determine the outcome or severity of infection. Among the three species, mice are relatively resistant to TB infection, followed by rabbits and then guinea pigs, which are extremely vulnerable to infection. Mice are most often used in experiments on immune responses to TB infection and drug regimens against TB. Rabbits, unlike the other two animal models, develop cavitary TB and offer a means to study the factors leading to this form of the disease. Guinea pigs, due to their high susceptibility to infection, have been ideal for studies on airborne transmission and vaccine efficacy. In addition to these three species, TB research has occasionally involved nonhuman primates and cattle models. Current concepts in TB pathogenesis have also been derived from animal studies involving experimentally induced infections with related mycobacteria (e.g., *Mycobacterium bovis*) whose manifestations in select animal hosts mimic human TB.

Keywords: tuberculosis; animal models; host/pathogen interactions

Although there are no naturally occurring animal reservoirs for *Mycobacterium tuberculosis*, many different animal species are susceptible to infection with this organism, transmitted unintentionally from humans, and transiently within certain other species (1–5). In addition, humans intentionally infect laboratory animals for experimental purposes. Soon after identifying the tubercle bacillus as the presumed cause of tuberculosis (TB), Robert Koch chose the guinea pig to demonstrate his famous postulates: that if an infectious agent is present in every case of a disease, and could be isolated from a diseased host, grown in pure culture, and cause disease when re-inoculated in an experimental animal model, it may be considered the cause of that disease. Since then the guinea pig has remained a valuable tool for the study of TB, and useful mouse and rabbit models have been added over the years.

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CLINICAL RELEVANCE

This review highlights the contributions of various animal models to the understanding of tuberculosis pathogenesis, host/pathogen interactions, and disease treatment, and discusses the reasons why each model is relevant to tuberculosis research.

LIMITS AND LESSONS FROM ANIMAL MODELS OF TB

Artificially infected guinea pigs, mice, and rabbits have served as indispensable tools through which transmission, immunopathogenesis, tuberculin response, vaccine and antimicrobial efficacy, genetic resistance, and many other important facets of tuberculosis have been studied. Results, however, are usually not entirely reflective of TB infection and disease in humans. Substantial differences in TB susceptibility, disease patterns, and temporal course exist among species (6–9) (*see* Table 1). The extent of organ involvement, immune response to aerosol or parenteral infection, and histopathology also vary considerably from species to species (6–9). In addition, a variety of clinical and laboratory strains of *M. tuberculosis* exist to infect animals experimentally, and these mycobacterial strains often differ greatly in infectivity, virulence, and immunogenicity in different animal models, as reviewed elsewhere (10). Well-defined host and pathogen variability allows researchers to control these factors, selecting those combinations needed to create animal models suited to the question being asked. Although infection by inhalation is the most relevant model for human infection, animal infections are also produced by parenteral inoculation. Like humans, tuberculosis in animal models is treated with antimicrobials given orally (by gavage) or by parenteral routes.

In addition to these considerations, animal species vary based on size, laboratory space requirements, rearing costs, and ability to approximate the disease process in humans. Below, we review key features of three of the most well-developed animal models of TB and then briefly discuss a few other, less commonly used animal species. Despite several important differences outlined in the sections that follow, the murine, rabbit, and guinea pig models have emerged at the forefront of TB research because (*1*) infection can occur with inhalation, (*2*) animals manifest an innate and acquired immune response, (*3*) animals often initially control bacillary growth in the lung, and (*4*) they ultimately succumb to the disease.

MICE

Mice are generally resistant to TB infection when compared with rabbits, guinea pigs, and even humans, as evidenced by their ability to tolerate relatively large bacillary numbers within their lungs (11) and other organs without signs of illness. Unlike humans, they develop noncaseating granulomata in response to

TABLE 1. COMPARING FEATURES OF COMMON ANIMAL MODELS OF TUBERCULOSIS

Model	Histopathology			Relative Susceptibility to *Mycobacterium tuberculosis*	Immunologic Reagents Available	Laboratory Space Requirements and Cost	Approximates Human Latent Tuberculosis Infection	Most Common Experimental Uses
	Necrosis	Caseation	Cavitation					
Mouse	Minimal; can depend on immune status	Usually not	No	Low	Extensive	Relatively small	No; Cornell Model may do so	Tuberculosis immunology; drug efficacy
Rabbit	Yes	Yes	Yes	Very low (*Mycobacterium bovis* typically used)	Moderate	Relatively large	No	Tuberculosis pathogenesis
Guinea Pig	Yes	Yes	Infrequent	Very high	Relatively few	Moderate	No	Vaccine efficacy; airborne transmission
Nonhuman primate	Yes	Yes	Yes	High	Extensive	Large	Yes	Tuberculosis pathogenesis; tuberculosis and retroviral immunodeficiency

infection, and rather than suppressing bacterial growth to a level consistent with latent infection, mice manifest a chronic phase of disease, in which immune-mediated tissue destruction occurs on a background of slowly progressive bacterial growth and results in death (8). This persistent stage of infection has helped investigators understand the effects of chronic exposure to mycobacterial antigens on T cell function, suggesting in part that CD4/CD8 T cell responses in the mouse remain robust over time (12). The murine immune response to TB has been carefully detailed using an extensive array of available antibodies and assays for cytokines and immune cells, more so than for any of the other animal species. As a result, researchers have also observed parallels between murine and human innate immune responses (13). For example, murine macrophages use Toll-like receptors on their cell surfaces (e.g., TLR 1 and 2) to recognize mycobacterial antigens and trigger cytokine production crucial to the granulomatous response (14–16). In addition, mice, like humans, demonstrate T cell–independent, natural killer cell production of IFN-γ, a cytokine crucial to the host immunologic response against TB, which may be relevant to understanding TB resistance in patients with HIV/AIDS with impaired T cell immunity (17). The availability of inbred and targeted genetic knockout strains of mice has helped elucidate the role of many specific cytokines, cells, and cell surface markers in containing bacillary growth. CD4 T cell deletions/knockout manipulations, for example, illustrate the central role these cells play in adaptive immune response to infection (18). In addition, among the animal models commonly used in TB research, the murine genome has been characterized and assembled in the form of a high-quality draft, permitting novel approaches to studying the host genetic contributions to fighting TB infection. The guinea pig and rabbit genomes have also been assembled and are being evaluated for deeper sequencing coverage, and can be found on the Broad Institute's website (www.broad.mit.edu/mammals).

Mice have been successfully infected by the aerosol route using whole body or nose-only exposure chambers, and acquire infection with relatively low doses ($\sim$ 50 colony-forming units [CFUs]) of *M. tuberculosis*. In the first 4 weeks after low-dose aerosol infection, mycobacterial growth is logarithmic and then plateaus around 10^6 organisms in the lungs when cell-mediated immunity (CMI) develops (19, 20). The plateau heralds the persistent stage of infection, in which mycobacteria may also be more metabolically quiescent within macrophages (7). As mentioned earlier, mice do not truly replicate paucibacillary, latent human TB infection. However, the development of the Cornell mouse model of TB arguably approximates latency or persistence through a drug-treated paucibacillary state of infection (21, 22). In the classic Cornell model (22), Webster-Swiss male mice infected intravenously with the H37Rv strain of *M. tuberculosis* were treated with oral isoniazid (INH) and pyrazinamide (PZA) for 12 weeks after bacterial inoculation. Antituberculous treatment reduced the number of bacilli in mice tissues for up to 3 months after cessation of INH/PZA, to the extent that mycobacteria could not be cultured or otherwise isolated from lung or other tissue homogenates (e.g., essentially "sterilized"). However, when observed for longer periods of time, about one third of similarly treated animals eventually spontaneously developed reactivation TB, characterized by a recrudescence of the bacterial burden in their tissues. To explore whether all "apparently sterile" animals at 3 months harbored dormant or latent organisms that could reactivate, researchers gave the mice high-dose immunosuppression. The results showed that appropriately timed steroids led to reactivation TB in most of these presumably "sterile" mice due to residual viable organisms, thereby approximating latency in the view of some but not all researchers. The development of this *drug*-induced latent TB model, albeit imperfect, provided an important adjunct to the conventional chronic TB disease model. Since the original description of the Cornell model, other investigators have manipulated a number of factors, such as the dose/duration of antibiotics, interval between antibiotic use and immunosuppression, and type of immunosuppression, to map specific cellular and cytokine mechanisms operative in the reactivation process (23). Taken together, the original Cornell model of latency and its subsequent variations have broadened the ability to probe the immunologic basis of an animal's ability to control bacterial replication *in vivo* and, perhaps, to demystify latent TB in humans. Although much debate surrounds the utility of the Cornell mouse model to accurately reflect human latency, it has also been adapted for use in some of the other animal species described below.

From a practical perspective, mice are generally easier to maintain in BSL3 facilities and offer a more affordable, high-yield means to study vaccines, antimycobacterial drugs, immune mechanisms, host genetics, and the contribution of host and pathogen strain differences leading to infection. Although it is still not known whether vaccines that are effective in mice would necessarily be effective in humans, the opposite holds true regarding TB drug studies in mice (13), where results have been, for the most part, reasonably predictive of the results of human clinical trials.

Before antituberculous medications or compounds can enter clinical testing, they undergo *in vitro* testing to determine their growth inhibitory potential, followed by pharmacologic testing for sterilizing activity in animals. Studies in mice are by far the most common animal model experiments that have examined the sterilizing activity of potential drugs, as well as the efficacy of shorter treatment durations using combinations of new and existing drugs. Recent evaluations have included regimens that substitute quinolone drugs (e.g., moxifloxacin) and a nitroimidazopyran such as PA-824 for isoniazid and rifampin (24), or studies that include rifapentine instead of rifampicin, taking advantage of rifapentine's longer half-life and resulting greater area under the curve (AUC), especially when administered daily or thrice weekly (25). For example, recent studies in the mouse model have shown that a regimen that incorporated daily rifapentine and moxifloxacin instead of rifampicin and isoniazid, respectively, resulted in faster bacterial clearance from murine lungs and lower relapse rates (25) compared with the standard 6-month regimen. Other work has evaluated newer compounds within a subclass of quinolones, the 2-pyridones, for efficacy compared with moxifloxacin (26) and found that one such compound's efficacy was better than INH, but not better than that of moxifloxacin.

Among the challenges in extrapolating the data obtained from mice drug studies are the ability to know how pharmacokinetic and pharmacodynamic parameters within the animal may relate to antibacterial activity in subsequent human studies. Improvements in the ability to more accurately establish serum drug concentrations and AUC values for drug concentration over time have enhanced the external validity of drug testing in these animal models (27).

Finally, in an effort to reduce the duration of animal drug studies, which can require several months to complete, some investigators have used selective gene knockout mice to accelerate the development of TB. One such example is that of the IFN-γ gene knockout mouse, called the GKO mouse (28). This mouse strain is more rapidly susceptible to *M. tuberculosis* infection and provides a faster, first line *in vivo* screening tool in which single drugs can be tested. Furthermore, because of the higher bacterial load achieved during infection, drug treatment in GKO mice has been shown to produce more substantial reductions in bacterial CFUs from the lungs compared with infected wild-type animals and after a shorter period of treatment (28). However, it must be kept in mind that absence of IFN-γ may alter elements of the functional host response to mycobacterial infection and result in changes in granuloma architecture, as recent work on granuloma necrosis in IFN-γ–deficient mice infected with *M. avium* suggests (29), with possible implications for drug penetration into such lesions in knockout mice compared with wild-type mice.

RABBITS

The rabbit model affords an opportunity to understand the pathology of TB infection by substituting *Mycobacterium bovis* infection as a surrogate for *M. tuberculosis*. However, the classic experiments of Lurie and Dannenberg clearly described TB pathogenesis in rabbits genetically inbred to be susceptible or resistant to airborne *M. tuberculosis* (30). Unfortunately, Lurie's genetically susceptible strain was allowed to die out, and currently available laboratory rabbit strains are relatively resistant to infection with *M. tuberculosis*. Established pulmonary infections with *M. tuberculosis* may form cavities, but they eventually regress and heal (31). In contrast, rabbits are significantly more susceptible to bovine mycobacterial infection with *M. bovis*, and the pulmonary pathology to inhaled bovine tubercle infection more closely resembles human *M. tuberculosis* infection than that seen in mice and guinea pigs (31). The rabbit is the only species of the three that easily develops pulmonary cavitation, resulting in bronchial spread of the pathogen. Substantial work has clarified the role that delayed type hypersensitivity (DTH) and CMI play in a rabbit's ability to form cavities after exposure to both whole tubercle bacilli or mycobacterial protein and lipid components (31–33). Understanding the mechanisms of TB cavity formation is particularly pertinent to transmissibility in humans for two reasons. First, pulmonary cavities harbor large populations (10^8) of bacilli that communicate with the bronchial tree, gaining access to the external environment. Second, human contagiousness correlates strongly with the degree of sputum culture positivity, reflecting bacillary burden. For these reasons, the rabbit, by reproducing cavitation, has greater potential for the study of disease transmission than other animal models.

Due to their previously mentioned resistance to *M. tuberculosis*, however, rabbits also serve as a model through which to study latent, or paucibacillary TB states in humans. In contrast to the Cornell mouse model, rabbits typically achieve a paucibacillary state through their own immune system's control of infection. However, unlike human latent infection, they tend not to spontaneously reactivate disease, unless they are experimentally immunosuppressed (6). Aerosol infection followed by steroid immunosuppression has also been used to study the effects of immune reconstitution on TB infection in these animals (6). Occasionally, it has also been observed that some rabbits that acquire infection and convert their tuberculin skin test, subsequently clear their infection, as judged by an inability to isolate bacilli from their tissues even after exogenous immunosuppression (6). There is evidence in early guinea pig studies (34, 35) and preliminary evidence from our own work with guinea pigs challenged with multidrug-resistant TB (our unpublished data, A.S. Dharmadhikari and E.A. Nardell) that arrested infection may also occur in the highly vulnerable guinea pigs, raising important questions about infectious dose, microbial virulence, and host defense.

Compared with mice and guinea pigs, rabbits are costlier to maintain and have larger laboratory space requirements. In addition, fewer immunologic reagents exists for rabbits than for mice. For many of these reasons, they are not as often used in TB research as are mice or guinea pigs.

GUINEA PIGS

Guinea pigs are well suited to study airborne TB transmission due to their exceptional vulnerability to infection with as little as a few inhaled mycobacteria (31, 36, 37). The guinea pig also replicates many aspects of TB infection in humans (especially childhood TB and TB in immunosuppressed hosts), including the formation of granulomata, primary and hematogenous pulmonary lesions, dissemination, and caseation necrosis. In addition, guinea pigs develop robust DTH (38). The classic experiments of Riley and colleagues, first proving that TB is an airborne infection, used guinea pigs as living air samplers for airborne tubercle bacilli generated by patients on an experimental TB ward. Infection was detected by DTH (tuberculin skin testing) and confirmed by both mycobacterial culture and histologic examination of tissues (lungs, spleens, and lymph nodes) (36, 37, 39). Guinea pigs have a larger minute ventilation than mice, so hundreds, not thousands, of animals were required to adequately sample air from the experimental ward. Together with colleagues, we have re-established a similar experimental ward in South Africa to investigate transmission and control of MDR-TB (40).

Similar to murine infection, the course of infection after aerosol challenge in guinea pigs consists initially of a logarithmic

phase of bacillary multiplication in the lungs over 2 to 4 weeks. After that, however, disease burden in the lungs enters a stationary phase due to DTH and CMI, while hematogenous dissemination to other organs occurs. Ultimately, hematogenous reseeding of the lungs occurs, adding to existing bacterial growth there, and progressive infection and tissue destruction (lungs, spleen) leads to demise (31, 41, 42). Experiments in which guinea pigs were either vaccinated or exposed to low-virulence *M. tuberculosis* isolates showed resistance to cavity formation and disease dissemination after reinfection with more virulent isolates, which was modified in some cases by malnutrition (42–44). Such studies are crucial for the development of improved vaccines, believed to be essential for TB control in endemic areas.

The guinea pig, however, lags the furthest behind among the above-mentioned animal models in terms of the availability of immunologic reagents for studying guinea pig host immune responses. Nonetheless, the numbers of reagents to study cytokines and other inflammatory cells involved in pathogen recognition and processing has been increasing, and promises to open new avenues of work with this animal model. Progress has been made in recent years in the cloning of guinea pig cytokine and chemokine genes, the expression of recombinant guinea pig cytokines and chemokines, and the use of these reagents in the study of the response of guinea pigs to infection with virulent *M. tuberculosis* (45, 46). Furthermore, using recently developed monoclonal antibodies for the guinea pig, other investigators have characterized the immune cell influx into lungs that occurs after low-dose aerosol infection and found that it consists of a large population of heterophil cells (47). In addition, they were able to characterize the specific cellular architecture and timing of the innate and adaptive pulmonary immune responses to low-dose infection and discovered that contrary to conventional understanding of this process, caseous necrosis was a relatively early event that occurred before the acquired immune response developed, and may in fact be attributable to the innate immune response (9).

OTHER ANIMAL MODELS

Nonhuman primates such as the cynomolgus macaque have also been successfully used to replicate human TB infection (48). This primate can be infected with low-dose aerosol concentrations ($\sim$ 25 CFUs) and manifests latent infection and reactivation as in humans. As shown in a recent study on a cohort of macaques challenged with low-dose aerosol, all animals demonstrated evidence of infection by either tuberculin skin test or lymphocyte proliferation assays to PPD, but only 60% subsequently developed active TB (49). Further investigation may soon shed light on the determinants of reactivation in humans. The nonhuman primate model also offers an opportunity to study the interaction between simian viral immunodeficiency (SIV) and TB as a model for human HIV/TB co-infection. Fortunately, cynomolgus macaque antigens cross-react with both immunologic reagents made for human cells and tissue as well as macaque-specific reagents, thus permitting immunohistochemical investigation into mechanisms of disease. Use of nonhuman primates for TB should, of course, be limited to questions that cannot be answered by lower species. The main disadvantages are cost and space requirements in BSL3 facilities. Because of the species' high susceptibility to TB and ability to horizontally transmit the disease, the potential for laboratory colony outbreaks has historically dampened enthusiasm for using this animal model. Horizontal respiratory transmission is much less frequent in rabbits, guinea pigs, and mice (5). Given all these factors, some investigators have advocated restricting the use of nonhuman primates to the final pre-clinical stages of vaccine or drug development trials (i.e., after testing in guinea pigs and mice) (50).

M. bovis infections in cattle have also been used to study the molecular mechanisms of TB infection. Bovine TB pathology is very similar to human TB in terms of granulomatous reactions and CMI, but differs with respect to cavitation (51, 52). Many immunologic reagents are available to study infection in this species. One of the new IFN-γ release assays being used to diagnose human *M. tuberculosis* infection was in fact developed to diagnose TB in cattle (53). Because of the parallels between bovine and human TB and the importance to the veterinary community of eliminating bovine TB, cattle field studies on BCG vaccine hold potential for advancing current understanding of vaccine immunology. The may also enhance our knowledge of the BCG vaccine's efficacy in human neonates because calves, like neonates, are immunocompetent at birth and develop protective immunity after BCG vaccination (52). As with the nonhuman primate, however, cattle studies are cumbersome to conduct.

FINDING THE RIGHT ANIMAL MODEL FOR VARIOUS TB DISEASE STATES

When humans are infected with *M. tuberculosis*, they may develop primary active TB, latent TB, chronic active TB, or reactivation disease. Not all manifestations are mutually exclusive, as 10% of nonimmunosuppressed individuals progress from latent to reactivation TB over their lifetimes, while HIV-infected individuals have a 10% annual risk of reactivating latent disease. Immunosuppression, HIV infection, nutritional status, intensity of exposure, BCG vaccination, and age determine, in part, individual outcomes. Less commonly reported, but of increasingly recognized importance, is the role that re-exposure to TB and re-infection play in the risk of developing disease (54). Each of these stages of infection in humans can be approached by the use of one or more of the aforementioned animal models.

CONCLUSIONS

For more than a hundred years animals have indeed taught humans a great deal about tuberculosis, and they promise to become increasingly useful as immunologic, genetic, molecular, and pharmacologic tools continue to evolve. Given the complexity of human tuberculosis, animal models of TB offer a vast resource to study a multitude of unresolved questions, ranging from the genetics of host defense, microbial virulence, latency, reactivation, reinfection, drug therapy, and immunization, to name just a few. Researchers are fortunate to have many well-developed experimental animal models from which to learn.

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