Animal models of tuberculosis for vaccine development

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Animal models for testing different vaccine candidates have been developed since a long time for studying tuberculosis. Mice, guinea pigs and rabbits are animals most frequently used. Each model has its own merits for studying human tuberculosis, and none completely mimics the human disease. Different animal models are being used depending upon the availability of the space, trained manpower as well as other resources. Efforts should continue to develop a vaccine which can replace/outperform the presently available vaccine BCG.

Key words Animal models - mycobacteria- tuberculosis - vaccines

Introduction

Tuberculosis (TB) caused by Mycobacterium tuberculosis is the second leading cause of mortality worldwide, especially in Asia and Africa. Globally 9.2 million new cases and 1.7 million death from tuberculosis occurred in 2002, of which 0.7 million cases and 0.2 million deaths were in HIV positive cases. If the same trend continues, one billion new people will be infected by 2020 and the disease will claim 35 million deaths. Presently, it is the leading cause of death in HIV infected patients in developing countries due to a high incidence of dual infections and decrease in the immunity against both HIV as well as M. tuberculosis infection. The emergence of increasing multi drug resistance (MDR) has further complicated the situation. The incidence of TB is on the rise up in East African countries followed by Eastern Europe as well (Estonia, Uzbekistan, Russia) where MDR TB represents 15 per cent of all TB cases and presents an ominous global threat.

Despite the use of TB vaccine Bacilli Calmette Guerin (BCG) under the aegis of WHO as well as directly observed chemotherapy programmes (DOTS), the TB pandemic has not abated. While DOTS had the potential to cure TB by 2005, the target of 70 per cent case detection with a cure rate of 85 per cent has only been achieved by few countries. In India, the Revised National TB Control Programme (RNTCP) had been recognized for the fastest expansion of DOTS in the world, with over 55-fold expansion in RNTCP coverage since 1998, leading to total coverage of the country in March 2006. But factors like failure or delay in diagnosis of the disease, moderate relapse rates (5%) after DOTS, long treatment duration to

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cure the disease have resulted in non-compliance and subsequent emergence of MDR *M. tuberculosis* strains\(^9\), present serious challenges to DOTS as well as RNTCP strategies. It is believed that for complete eradication of TB, a cheap and effective vaccine is required for mass immunization\(^10\). BCG (live, attenuated *M. bovis*) is the only vaccine presently available against tuberculosis but it can prevent only an estimated 5 per cent of the all potentially vaccine preventable deaths due to tuberculosis\(^11\). Further, it has been found to be effective against disseminated and meningeal tuberculosis in young children\(^12\). The protective efficacy of BCG vaccine against adult pulmonary TB has varied from 80 per cent in UK to 0 per cent in Chinglepet, India\(^13,14\). At present about 100 million children receive BCG vaccine each year. Although the oldest of the currently used vaccine, BCG is still controversial in that there are conflicting data on its protective efficacy\(^15\).

There is no doubt that we need to have an effective vaccine for the ultimate control of the current TB pandemic\(^16\). During the last few years extensive work has been done and more than 200 candidate vaccines have been evaluated using different animal models. All this has been extensively reviewed\(^17,18\). Clearly, the development of new and more effective vaccines and immunization strategies designed to protect against primary infection and to boost waning BCG induced protective immune responses are needed for worldwide control of tuberculosis.

**Animal models: Contributions in TB vaccine testing**

Animal models have played an important role in the testing of different vaccine candidates. Due to the striking similarities between human and animal physiology, they provide valuable information about the human systems. The use of animal models to TB research has a long history since Robert Koch first used mouse as an experimental model\(^19\). Subsequently, investigators established infections in a variety of animal models (rabbits, guinea pigs, rats, monkeys, *etc.*)\(^19\). Animal models of pulmonary tuberculosis in the mouse and guinea pig are being extensively used and provide new information about the host response in the lungs, changes in immunopathology and the protective effect of new vaccine candidates throughout the world\(^19\).

Though each model has advantages and disadvantages and the results from them can be extrapolated to humans, these models resemble one or the other facets of human disease. First and the foremost advantage of using these models is that these animals can be easily infected by pulmonary route as a result of few virulent tubercle bacilli get deposited in alveolar space in the same way humans acquire infection. Further, it is easy to study various stages of TB progression like granuloma formation, liquefaction, cavity formation and haematogenous spread of the disease in animal models (except mice)\(^21\). The symptoms of the disease like fever, loss in weight, abnormal X-rays and respiratory distress can also be seen in these models. The animals eventually die of pulmonary insufficiency if left untreated, like the human patients. Because of these similarities in animal models and the humans in the susceptibility as well as resistance to TB, disease progression and finally death, the animals are considered good models for evaluating new anti-TB vaccines as well as new anti-tubercular compounds.

### Mouse model

Mouse is one of the most popular and economical experimental model used since the time of Robert Koch. Mice can be easily infected via aerosol with a low dose of organisms, multiplying in lungs and subsequently spreading to liver and spleen. The infection is controlled but not eliminated, by cell-mediated immunity, mainly T cell responses. The resulting infection is well tolerated for more than one year in some strains of mice\(^20\). The immune response to mouse has been shown to have direct correlates in the human system, including the importance of CD4T+ cells\(^21-23\), interleukin-12 (IL-12)\(^24,25\) and tumour necrosis factor-α (TNF-α)\(^26-29\).

The subcutaneous and intravenous routes were popular for infecting the animals prior to the discovery of aerosol inhalation chamber. Presently, the low dose aerosol is being used in the pre-clinical screening of vaccines. The mouse model of C57BL/6 as well as BALB/c strains are well characterized and the tuberculosis mice survive two times than mice of DBA/2 and C3H/HeJ strains\(^30\). In this approach, mice are immunized, rested for a short duration of 30 days and subsequently challenged with a low dose of aerosol of virulent *M. tuberculosis* which can multiply in the lungs and spread to other organs, most notably spleen and the liver. After an interval of 4 wk, the mice are sacrificed and number of colony forming units (Cfu) in lungs and spleen are enumerated by plating on...
The major advantage of murine model for vaccine development lies in its ability to screen a large number of vaccines at a limited cost and to determine how the vaccine mediates any protective effect. This model has been extensively used for evaluation of candidates vaccines. Further, this model is helpful in determining how the route of immunization, type of adjuvant and specific antigen affect the ability of memory T cells to accumulate at the site of the challenge. A better understanding of these aspects of vaccine induced protection plays an important role in designing the effective vaccines.

The point which goes against using murine model is nature of protection as the results may not be extrapolated directly to the human beings. The process of granuloma formation in mice after TB infection is altogether different from that seen in humans as well as other naturally susceptible hosts (e.g., guinea pigs) due to its innate resistance to the tuberculosis and generation of strong cellular responses against TB infection which in turn, controls bacterial growth as well as progression of the disease. There are many studies which describe that granuloma in mice characterized with the aggregation of lymphocytes towards center while in humans and guinea pigs, lymphocytes form a peripheral ring with macrophages placed in the center. Therefore, mouse model has an edge in immunological studies of TB and hence found suitable for the first order screening of vaccine candidates and the efficacy of new candidate vaccines, and those showing good protection in mice are then evaluated in other animal models of tuberculosis.

Guinea pig model

Guinea pigs are extremely sensitive to infection with *M. tuberculosis* and this model provides an important device for identifying the effective anti-tuberculosis chemotherapy, vaccines as well as the potential role of mycobacterial constituents as virulence factors. This model has been well characterized and it is possible to efficiently infect the animals by aerosol route with a small number of bacilli. The invariably fatal course of disease progression in this model provides a reliable parameter for studying the protection by a candidate vaccine. Though in the mouse model, only ~ 1 unit log reduction in peak lung bacillary load is achievable by BCG vaccine, the reduction is 2-3 log unit in immunized guinea pigs which provides a wider spectrum for evaluating the efficacies of the vaccine candidates.

For vaccine development, reduction or prevention of tissue damage is an important criterion which can be easily assessed in this model. This model is similar to the mouse model but the experiments are open ended survival type and may go up to 2 yr. Guinea pigs are peculiar as they develop classical granuloma similar to humans. Other important features of this model is the presence of Langhans multinucleated giant cells. These cells are formed due to fusion of macrophages and can often be seen within human granulomas. With progression of active disease, the guinea pigs develop lung tissue necrosis, start losing the weight and die due to the disease like humans. Thus, guinea pig granuloma exhibits many characteristics like the humans. A major difference from humans is the inherent susceptibility of guinea pigs as majority of the infected human beings can contain the TB infection.

Most of the vaccine candidates are first evaluated in mice model and the promising ones are subsequently taken up for evaluation in guinea pig model. But this strategy should be cautiously used as there is a possibility of loosing some good candidates which may not be promising in mice model but show good protection in guinea pigs which resemble more to the humans. Though guinea pig model of TB has several advantages, it suffers from some disadvantages which precluded its use as first order screening model. These are (i) high rearing cost of animals in a biosafety facility compared to low expenses involved in housing mice, and (ii) limited availability of reagents to assess immunologic factors involved in protection in vaccine studies though efforts are being made to develop these reagents.

Rabbit model

In TB infected rabbits, the lung tissue is destroyed largely as a result of the host’s own reaction to bacillary antigens. The rabbit is being used as a model of human tuberculosis since the beginning of 20th century because of its innate resistance to tuberculosis as well as its close resemblance to human TB. This model has been extensively used by many groups. It has also been used for addressing a number of questions related to tuberculosis. In the era before genomics
and before differences in biochemical properties were elucidated, the rabbit infection model was used to differentiate between \textit{M. bovis} and \textit{M. tuberculosis} because of the remarkable difference in virulence of \textit{M. bovis} and \textit{M. tuberculosis} for these animals\textsuperscript{54}. Rabbits have also been used to examine the difference in relative pathogenicities between \textit{M. tuberculosis} strains CDC 1551 and H37Rv. Tubercles from animals infected with CDC 1551 were smaller and contain lesser bacilli than those infected with H37Rv\textsuperscript{48}.

The rabbit model offers certain advantages over murine and guinea pig models. First, when infected with \textit{M. tuberculosis} or \textit{M. bovis}, rabbits have a spectrum of disease that represents many of the specific stages of human disease. In general, rabbits are able to contain disease caused by virulent \textit{M. tuberculosis}. Over time, the number of pulmonary bacilli declines and lesions regress\textsuperscript{49}. With \textit{M. bovis} infection, rabbits form chronic fibrosing pulmonary cavities\textsuperscript{50}. Both of these are prominent features of human disease. Finally, rabbit granulomas, with their caseous centers, closely resemble the human granuloma. Lurie reported a remarkable similarity between the spectrum of rabbit tuberculosis and that found in humans\textsuperscript{49}.

The outbred rabbits are known to be relatively resistant to intravenous and aerosol infection with \textit{M. tuberculosis} and generally recover from infection in 4-6 months like humans\textsuperscript{57,59}. In general, they are able to contain the disease. On the other hand, the inbreds animals are more susceptible to TB than their outbred counterparts and had an impaired ability to contain the disease\textsuperscript{50}. When inbred and outbred animals were compared histologically, inbred animals showed more caseous necrosis, more visible bacilli and fewer epitheloid cells, suggesting that inbred animals were more susceptible to TB than the outbreds. More grossly visible and larger tubercles could be seen in the inbred animals than the outbred animals\textsuperscript{52}.

There are limited reports where rabbit model has been used for the evaluation of the candidate vaccines. Immunomodulators like live attenuated vaccines (\textit{M. vaccae}) have been tested in the aerosol cavitation rabbit model but no statistically significant differences in the number of cavitary lesions or tuberculin responsiveness could be detected\textsuperscript{50}. Recently Tsenova and coworkers\textsuperscript{58} used this model of tuberculous meningitis for vaccine studies. Vaccination with Mtb72F (polypeptide formulated in AS02A (Mt 72F+AS01B) showed protection against central nervous system (CNS) challenge with \textit{M. tuberculosis} H37Rv to an extent comparable to that of vaccination with BCG\textsuperscript{58}. Similar accelerated clearances of bacilli from cerebrospinal fluid, reduced leukocytosis, and less pathology of brain and lungs were also noted. In addition, protection against \textit{M. tuberculosis} H37Rv CNS infection afforded by BCG/Mtb72F in a prime-boost strategy was similar to that by BCG alone\textsuperscript{59}.

The rabbit model like other animal models, has some limitations, including the paucity of commercial immunologic reagents. In addition, inbred rabbits in large numbers are not available, consequently experiments are done in outbred animals, giving rise to larger variations in results within each vaccination group. Nevertheless, this diversity would also be the case when any vaccine is tested in humans. However, with outbred animals, a large group of 15-20 animals will be required to demonstrate significant advantage of a new vaccine over the BCG.

\textbf{Non-human primate model}

The non-human primate models of tuberculosis have a long history and are being used for many years for vaccine and drug testing studies\textsuperscript{59,61}. The disease in monkeys is usually a progressive pulmonary disease with both haematogenous and bronchial spread of the bacilli. Further, extensive caseous necrosis along with liquefaction of the caseous material with cavity formation has also been reported\textsuperscript{59,62-66}. The severity is reduced when monkeys are immunized with BCG\textsuperscript{63} which can prevent visible pulmonary lesions in some animals if only a few virulent \textit{M. tuberculosis} are initially inhaled. For tuberculosis studies, both Rhesus (\textit{Macaca mulatta}) and cynomolgous (\textit{Macaca fascicularis}) monkeys have been used. Although it is possible to infect monkeys by aerosol\textsuperscript{59,64}, specialized equipment is necessary and standardizing a dose delivered to the monkey is difficult. Walsh \textit{et al}\textsuperscript{68} reported that cynomolgous macaques when infected with lower doses of \textit{M. tuberculosis} (10-100 Cfu), a few bacilli/or no clinical symptoms were seen, however, a larger inoculum (3000 Cfu) caused progressive disease in all the monkeys\textsuperscript{58,66}. Capuno \textit{et al}\textsuperscript{58} demonstrated that cynomolgous macaques could be infected with very low doses of \textit{M. tuberculosis} delivered to the lungs via flexible bronchoscope. These animals when observed up to 15-20 months showed the spectrum of the disease as seen in humans.

Because of their phylogenetic relationship with humans and their well characterized immune system, macaques are often used to evaluate the immunogenicity
and safety of new candidates. Further, macaques are susceptible to M. tuberculosis and develop a course of infection that clinically and pathologically resembles that of M. tuberculosis. It may also be important that several of the host molecules implicated in TB infections are present in man and non human primates but are not present in mice and guinea pigs. Studies have indicated that Rhesus macaques are highly susceptible to tuberculosis, the closely related cynomolgous macaque being more resistant. Moreover, the cynomolgous monkeys are more efficiently protected by BCG vaccination than Rhesus monkeys and therefore offer a good experimental model for evaluation of new subunit vaccines where protection would be measured in terms of the ability to reduce symptoms, pathology and bacterial load.

In recent times, scientists have shown interest in non human primate models for testing vaccine candidates and vaccine candidates have shown good protection in these models.

Though monkeys are costly, yet they can be a useful model for evaluation of the candidate vaccines that have shown promising results in other models. Any candidate showing good protection would stand of providing infection in humans.

The non-human primates have an edge over other models because of similarity to human tuberculosis, spectrum of disease and pathology as well as availability of the reagents for studying the immunological parameters. This translates into obtaining results that are more directly applicable to the human situation. However, these models have some disadvantages like high cost, biosafety facilities as well as non availability of inbred animals. Though the outbred animals may cause more variability in the experiments, they are more akin to the human population. Moreover, monkeys with tuberculosis are contagious to other animals, including other monkeys and the laboratory personnel posing a serious risk in an animal facility. The high cost involved in non-human primate research is due to the cost of the monkeys, need for veterinary care and veterinary technicians on a regular basis and a large space needed per animal. Although the non-human primate model appears to be an attractive model for tuberculosis research but these factors limit the use of this model in many research institutions.

Cattle model

During the last 15 years, there has been extensive research on bovine tuberculosis and scientists are making serious efforts for understanding the pathogenesis of disease, developing better diagnostic tools and vaccines for disease control. Unlike the humans, cattle can be experimentally challenged which results in a reproducible disease and the trials can be completed within a relatively short time. The advantages of working with cattle model include the following: (i) the experimentally induced disease is studied in the natural host with infections acquired predominantly via the respiratory route which helps in the meaningful screening of the vaccines, (ii) the clinical disease may take years to develop, (iii) the disease has identical pathology in terms of granulomatous reactions and immune responses to that in humans, (iv) availability of a large number of immunological reagents, (v) calves being immunologically competent at birth, neonatal vaccination is possible, (vi) calves being sensitized to antigens of environmental mycobacteria at younger age like humans, and (vii) BCG has variable efficacy in cattle like humans which provides an opportunity to detect better vaccines than BCG. The main disadvantages of this model are (i) instead of M. tuberculosis, this model uses M. bovis, (ii) absence of cavitation like humans, and (iii) high cost involved in rearing the cattle.

A variety of the tuberculosis vaccines like attenuated M. bovis vaccine strains, DNA vaccines as well as protein vaccines have been tested in cattle vaccination/challenge model. However, tuberculosis protein vaccines have not yielded the desirable results in cattle model. A major limiting factor in the development of effective protein vaccines is the lack of appropriate adjuvants which can induce strong cellular responses in cattle. However, neonatal calves are a good model for testing vaccines in infants since they are immunocompetent at birth and become naturally sensitized to environmental mycobacteria at a younger age.

Conclusions

Though different animal models are in use for long for tuberculosis research providing valuable information, none completely mimic the human model. However, refinement of animal models may pave the way to new information of great importance. No single model is good enough for vaccine evaluation and the choice of model is mainly dependent on cost, availability, space as well as biosafety requirements.
References


