Review article

Guinea pig model of tuberculosis

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Abstract

Animal models are being developed for testing different vaccine candidates as well as testing of new antitubercular since a long time. Mice, guinea pigs and rabbits are animals which are frequently used. Though each model has got its merits as well as demerits and each of them differ from human tuberculosis in one aspect or the other but none of the model completely mimics the human disease. Out of the different animal species, guinea pig model is one of the better models as it is very sensitive to *M. tuberculosis* infection but it has certain limitations like paucity of immunological reagents. However, it is the best model for tuberculosis research.

Keywords: Vaccines; Animal Models; Tuberculosis; Guinea pig

INTRODUCTION

In the 19th and 20th century, the guinea pig (*Cavea porcellus*) was the most popular experimental model for studying the infectious diseases like tuberculosis and diphtheria. By using guinea pig model, Robert Koch could develop the postulates of infectious disease etiology, essential for identifying the causative agent of infectious disease[1,2]. He also used guinea pigs in his classical experiments establishing that Mycobacterium tuberculosis as the causative agent of tuberculosis. The recent taxonomical studies have changed its position from class rodents to non rodents[3,4]. This is a better representative model of human infection than mouse[5,6]. Sisk[7] narrated number of similarities between humans and the guinea pigs which have direct or indirect bearing on the relevance of guinea pig as a model of infectious disease. The two species resemble with each other with regard to hormonal and immunological responses[8-11], physiology of pulmonary tract[12,13], corticoid resistance[14], need for exogenous supply of Ascorbic acid[15,16] and delayed type hypersensitivity (DTH) after exposure to infection[11].

Tuberculosis is one of the most important bacterial disease that has been well characterized[11, 17]. For creating this model, the animals are exposed to a small number of bacilli (10-50CFU) by aerosol route, a situation resembling human transmission. Extensive research conducted with this model indicates that the guinea pig is good model for primary human tuberculosis due to its extreme susceptibility to the infection, resembling symptoms and pathology, DTH response, good recovery after standard oral chemotherapies and excellent protection against tuberculosis (TB) after immunization with Bacille-Calmette Guerin(BCG) vaccine[11, 17-19]. Lymphadenitis is also common in guinea pigs like children infected with tuberculosis bacilli[20]. This model has also helped in understanding the effects of malnutrition which is a risk factor among the human populations. The protein malnourished guinea pigs infected with mycobacteria showed many immunological deficits like loss of protection following BCG vaccination[21-24]. However, contrary to the humans, the liquefication and cavitation of pulmonary granulomas within infected lung tissues are rarely seen in guinea pigs[11, 18] and the animals never show the latent form of the disease[25,26]. During last 50 years, this model

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has facilitated in understanding the fundamental relationship between the TB bacilli and the human host. The early studies on pathogenesis of experimental tuberculosis in control and vaccinated guinea pigs showed that the previous exposure of animals to mycobacterial antigens led to delayed type of hypersensitivity resulting in lesser accumulation of the bacilli in the lung tissues[27,28]. The interaction between tubercle bacilli and host phagocytic cells [29] and potential role of the mycobacterial constituents as virulence factors have also been studied in guinea pig model only[30,31].

Guinea pig model has been extensively used in testing the biological reagents as well as drugs against tuberculosis. Guinea pigs respond quite well against anti-TB antibiotics and hence widely used to test the new drugs or the drug combinations[32,33]. With the increasing number of multi drug resistant strains of *M. tuberculosis*, this model will be of immense importance in search for new and efficient anti-mycobacterial drugs[34]. PA-824, the most promising new drug candidate of recent time, was found to be efficacious in guinea pig model of low dose pulmonary infection[35]. The tuberculosis infected guinea pig has been considered the 'gold standard' for preclinical testing of the novel drugs and candidate vaccines[36,37], various methods of delivery and the biological standardization of tuberculins for use in human skin testing[38,39]. There is a need to develop improved drug regimens and preventive vaccines as the administration of present chemotherapeutic agents cause hepatotoxicity and poor patient compliance[40]. Some studies have also been conducted so as to evaluate the aerosolized administration of the anti-tubercular drugs rather than the oral route in guinea pig model[41-43]. The results from these studies indicated that this mode resulted in reducing the bacillary load in the lungs. BCG is presently the only vaccine available for prevention against tuberculosis. However it is highly controversial due to variable protective efficacy in different human populations[44-46]. Hence this model has been tried to test various novel adjuvants and vaccine testing . Many type of vaccines have been tested in this model including non-viable bacilli[47] attenuated mycobacteria[48], recombinant BCG strains[49-53], recombinant proteins /peptide vaccines[54-55], DNA vaccines[56-58], auxotrophs and gene-knockout mutants[59-63] and other mycobacteria[64]. The guinea pigs exhibit DTH response against these vaccines which is measured by skin induration test after intradermal injection[11,65].

Besides the primary pulmonary tuberculosis, this model has helped in understanding the other forms of the disease like tuberculous pleuritis, exogenous re-infection and endogenous reactivation. Intraplural injection of either BCG[66] or heat killed *M. tuberculosis*[67,68] induced pleuritis in guinea pigs. Exogenous re-infection has also been studied in guinea pigs but the re-infection by pulmonary route with virulent *M. tuberculosis* of guinea pigs previously infected with non- tuberculous mycobacteria (NTM) or low virulent clinical isolates failed to exacerbate the disease like the humans[69] but showed protection[70,71]. The re-infected animals responded better to the second challenge of virulent bacilli than those infected for the first time under uniform conditions . Further the protective effect of a prior pulmonary exposure to a low virulent isolate was greatly impaired by protein deficiency[72]. Guinea pig model of endogenous reactivation will help us in better understanding the factors associated with persistence of tuberculosis bacilli in tissues as well as the events that convert the dormant to active mycobacteria and allow their multiplication in large number. Though limited data is available on reactivation of tuberculosis in guinea pig model, Smith and Wiegeshaus[73] has described the protocol for development of this model.

Besides the good model for testing the new candidate vaccines and new anti-tubercular drugs, this model has been helpful for elucidation of the immune response to infection and the basis for protective effects of the BCG. Number of scientists have reported that due to lack of the immunologic reagents for guinea pigs, adequate immunological studies can not be made in tuberculosis infected guinea pigs[11,18,25,36,74]. Just to tackle this situation, numerous attempts have been made by the scientists to perform bioassays[75,76], develop recombinant cytokines[77-81] as well as develop antibodies[80-82] and antiserum[81] against these immune-mediators. Number of molecular techniques like Real time PCR for determination of cytokine and chemokine m-RNA levels[83-85], semi-quantitative PCR for RNA extraction[86,87] and Southern and Northern blot analyses for studying gene expression[88-90] and Microarray - mRNA expression data[91] have further helped in

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better understanding of the immunology of tuberculosis in M. tuberculosis infected guinea pigs .

Inspite of all the recent developments , the guinea pig models suffers from : (i) Lack of readily available immunological reagents required for qualitative and quantitative evaluation of immune responses; (ii) High cost involved in rearing guinea pigs under BSL-3 conditions ; (iii) Requirement of good husbandry practices needed for rearing this species. However, the biological relevance of this species outweighs these limitations and efforts are needed to improve this model further.

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