

Contribution of Animal Model Research Unit- towards pre-clinical vaccine trials

Background: Creation of Animal House Facility at NTI

In 1962, after establishment of Bacteriology Section, proposal for creation of Animal House facility at NTI was submitted to the Government of India. It took 15 years before CPWD could hand over the building to NTI in 1974. Just after its establishment, the section chalked out the plan to further experiment in Laboratory Animals and their utility in TB Control. In 1979, Breeding wing became operational and breeding of laboratory animals, mainly albino guinea pigs, was started and maintained thereafter, ensuring homogeneity of the successive generation of the stock animals. In 1981, preliminary in-vivo tests started on a modest scale not only to get familiarized with the procedures, but also to standardize various techniques.

From 1983 onwards, various research projects involving experiments in the animal model including collaborative research with other organizations were undertaken viz., (a) Virulence of tubercle bacilli isolated from pulmonary and extra-pulmonary tuberculosis (TB meningitis) patients in and around Bangalore. (b) Role of non-tuberculous mycobacteria (*M.avium-intracellularae*) in immunization against TB. (c) Behavior of south India variant of *M.tuberculosis* on a serial animal passage in albino guinea pigs. (d) Haemotogenous dissemination of pulmonary isolates of *M.tuberculosis* in Animal Model – A Quantitative Measurement. (e) Drug sensitivity pattern and virulence status of *M.tuberculosis* growth in the presence of 10% Carbon-Di-Oxide. (f) Evaluation of recombinant DNA vaccine against TB in Animal Model in collaboration with the Department of Microbiology and Cell Biology, Indian Institute of Science (IISc), Bangalore. (g) The pathogenetic role of serine threonine kinases of *M.tuberculosis* in albino guinea pigs- A collaborative study with Tuberculosis Research Centre (TRC) Chennai.(h) Virulence assay of parental, dev R mutant and complemented strains of *M.tuberculosis* in NTI-bred albino guinea pigs- A collaborative study with the Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), New Delhi.

Highlights from some of the studies conducted at NTI

- Study on virulence of tubercle bacilli in guinea pig animal model revealed isolation of high virulent tubercle bacilli in significant number of TB meningitis patients' cerebro-spinal fluid in contrast to sputum specimen from pulmonary TB patients where majority of them excreted low virulent tubercle bacilli. Implications from the findings indicate need for new strategies in the development of vaccine for prevention of TB¹.

In another study carried out between 1985-1988, isolates of *M.tuberculosis* from urine and sputum specimens of pulmonary tuberculosis patients were found to differ in their virulence status, phage type and drug sensitivity pattern, in a significant number of cases. The study indicated perhaps for the first time that a number of bacillary pulmonary tuberculosis cases resulted from exogenous re-infection instead of endogenous re-activation TB. This finding prompted for further investigation to understand the key events in the pathogenesis of TB leading to the development of cavitary lesions with a scope for innovative & effective intervention².

- Low virulent character of tubercle bacilli isolated from most of pulmonary TB patients in south India, termed as “South Indian Variant” was interestingly found to remain unchanged during eight years of serial animal passage in albino guinea pig animal model³.
- Studies on evaluation of protective efficacy of BCG and role of environmental mycobacterial infection in immunization against TB revealed possible explanation for the lack of protection from BCG in the Tuberculosis Prevention Trial conducted in Chingleput district of Tamil Nadu⁴. These studies were followed by improvisation for assessing the haemotogenous dissemination of tubercle bacilli in the guinea pig animal model by the quantitative recovery of tubercle bacilli for the target organ (spleen) of the albino guinea pigs. Based on its reproducibility in the experiments conducted at various laboratories irrespective of the variation in scoring the gross disease (tuberculous lesions) by different investigators, the technique has become quite useful not only in virulence assay but also in evaluation⁵.
- “Evaluation of Chemotherapeutic potential of polymer entrapped isoniazid, rifampicin and pyrazinamide V/s free drugs in guinea pigs” in collaboration with Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh. Despite effective chemotherapy of tuberculosis, patient compliance remains the single most important reason for the failure of treatment in addition to invariable side effects associated with free drug administration. To overcome these problems, depot preparations of anti-mycobacterial drugs such as Isoniazid, Rifampicin and Pyrazinamide have been effectively used and synthetic biodegradable poly (lectide-co-glycolide) co-polymers (PLG) are among the primary candidates because of excellent tissue bio-compatibility, bio-degradability and regulatory approval. Chemotherapeutic efficacy and sustained bioavailability of Rifampicin and Isoniazid in biodegradable form were further studied at PGIMER, Chandigarh.
- “Screening two novel *M. tuberculosis* proteins for human T-cell response and testing DNA expressing the two proteins in guinea pigs for protection against tuberculosis” in collaboration with Centre for Genetic Engineering (CGE), Indian Institute of Science (IISc), Bangalore. Under Prime Minister’s “JAI VIGYAN” programme for research into vaccines for several infectious diseases afflicting India, DBT funded study has been undertaken. As an alternative to immunization with purified human sputum isolate of *M. tuberculosis* (NTI-64719) was taken for obtaining DNA with extensive dissemination in guinea pig model and used to construct an expression library in the Lamda vector at IISc, Bangalore. Out of 176 recombinants probed, interestingly, some recombinants did not react with early phase of serum in TB patients. One of the major achievements so far has been finding a recombinant protein that elicited good proliferative response in healthy contacts of TB patients. The initial phase of above study has been published in an international journal⁶.
- “Virulence assay of parental, dev R Mutant and complemented strains of *M. tuberculosis* in NTI-bred albino guinea pigs”. A collaborative study with the Department of Bio-Technology, All India Institute of Medical Sciences (AIIMS) New Delhi. With the recent understanding of genome sequence of *M. tuberculosis*, efforts to combat tuberculosis have intensified. Various mutant strains and the devR gene, constructed at AIIMS along with other complemented strain of *M. tuberculosis* are being studied for their virulence. In view of their potential for developing better vaccines and contribution in the identification of drug targets active against quiescent/persistent tubercle bacilli.

- The IAEC approved collaborative project has been undertaken with the objective to evaluate and compare virulence of *M. tuberculosis* wild type, devR mutant and complemented strain in NTI bred albino guinea pigs. The study was initiated to understand the role of mutant/complemented strains in the guinea pig model of virulence. The findings from first phase of experiments showed virulence of dev-R mutant strains marginally less than H37Rv, while the complemented strain was significantly attenuated (under publication). There was no effect of passaging in the complimented strain, making it ideal candidate for further investigation which will pave the way for development of better vaccine and newer drugs active against persistent TB.

Animal Model Research Unit (AMRU) at National Tuberculosis Institute (NTI), Bangalore is registered under Breeding of & Experiments with the Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice & Empowerment. Studies involving animal experimentation are subject to approval by the Institutional Animal Ethics Committee (IAEC) constituted as per the prescribed guidelines, which includes a nominee from CPCSEA. In this regard, all the guidelines of Government of India are complied with. In view of advancement in TB research, modernization and 'facility safeguards' a Bio-Safety Laboratory (negative pressure) facility & an open shelter for retired breeders have been established at NTI.

References

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