CHAPTER 6

SUMMARY AND CONCLUSIONS
The work reported in this thesis centers on the immunizing potential of a cultivable, nonpathogenic mycobacterium, *Mycobacterium w*, which shares *B* and *T* cell antigens with *Mycobacterium tuberculosis*. *M.w* is a fast grower and was identified from amongst fifteen mycobacteria for its ability to evoke immune reactivity in lepromatous leprosy patients. A vaccine based on killed *M.w* is approved for human use and is currently in large scale phase III trials in leprosy patients and their household contacts in two hospitals and an endemic field area in India. BCG has shown low degree of protection against tuberculosis in the large scale trials conducted in South India under the auspices of Indian Council of Medical Research, World Health Organisation and other agencies. There is need to develop a vaccine with higher protective potential against tuberculosis. BCG in live state, but not in killed form, immunizes some genetic strains (BALB/c By J and C57BL/6 N Crl) of mice but it is a poor immunogen in other strains (C3H/He N Crl and CBA/N). The initial studies were carried out to determine whether *M.w* can protect those strains of mice where BCG demonstrates poor immunizing potential. It was further considered of interest to determine whether the efficacy of *M.w* is genetically restricted in the way observed for BCG. The early experiments demonstrated the protective immunizing capability of *M.w* in killed form in contrast to live bacilli mandatory for BCG action. The vaccine employed
for the studies was thus a suspension of autoclaved M.w suspended in saline. It was administered by the s.c. route. A summary of experiments and results follows.

1. Four strains of mice, two of which are known to be responders to BCG, namely BALB/c ByJ, C57BL/6 NCrl (BCG-susceptible, Bcg⁰) and another two known to be non-responders to BCG i.e. C3H/He NCrl and CBA/N (BCG-resistant, Bcg¹) were experimentally infected with M.tuberculosis H37Rv to induce a sub-lethal infection. The level of infection was assessed by screening the mice for tuberculin reaction, pulmonary lesions, and viable units of mycobacteria recovered from the lungs, spleen and liver. On prior immunization with 1x10⁷ heat killed suspension of Mycobacterium w, an anti-leprosy vaccine currently under large scale human trials in India, all the four strains of mice used in the study were protected against tuberculosis as indicated by a significant reduction in pulmonary lesions and viable units of mycobacteria. On infection with M.tuberculosis H37Rv, 90% of the animals in the control group of mice developed pulmonary lesions. Mycobacterium w was able to reduce the percentage of animals with lesions to approximately 18% in Bcg¹ (CBA/N, C3H/He NCrl) and 10% in Bcg⁰ (BALB/c ByJ, C57BL/6N Crl) strains of mice. Although, a few animals in M.w. vaccinated group did develop lesions, the number of organisms found in their tissues was distinctly lower than that in unimmunized
animals. Approximately 8 to 10 fold reduction in the number of culturable units of H37Rv in the lung, liver and spleen was obtained in all four strains of mice immunized with M.w. Results of these experiments suggest that a vaccine based on heat killed *Mycobacterium* w has the potential to confer protection against tuberculosis even in those strains of mice which are not protected by the currently employed live BCG vaccine.

2. In parallel experiments, live BCG was able to confer protection only to mice of Bcg^S^ strains but not to mice of the Bcg^R^ strains. The efficacy of M.w vaccine in Bcg^S^ strains was comparable to that of BCG.

3. Experiments were also carried out to study the protective effect of M.w against tuberculosis in guinea pigs, an animal species known to be exquisitely susceptible to experimental infection with *M.tuberculosis* H37Rv. A sublethal dose of 1x10^5^ of *M.tuberculosis* H37Rv was given intra-muscularly in the thigh and the course of disease was followed by skin testing with purified protein derivative and by autopsy at four weeks post-infection. Development of progressive disease was evidenced by gross tubercular lesions in the lungs and spleen after four weeks and CFUs of *M.tuberculosis* H37Rv recovered from these organs. Immunization with 5x10^8^ M.w s.c., and then challenge by virulent *M.tuberculosis* H37Rv significantly
reduced the course and severity of disease as evidenced by absence of gross lesions as well as considerable reduction, of the order of 20 to 30-fold, in the number of CFUs recovered from different organs. The degree of protection conferred by M.w was similar to that by BCG.

4. In the next series of experiments, the mechanism underlying the protection was studied. The aim was to investigate the role of T cell enriched lymph node cells in protective immunity as also the effect of M.w immunization against a nonspecific challenge by Listeria monocytogenes. One BCG-nonresponder strain of mice i.e. C3H (Bcg^r) and another BCG-responder BALB/c (Bcg^s) strain of mice were used in these studies.

For adoptive transfer studies, donor animals were immunized s.c. with 1x10^7 M.w. On day 21 post-immunization, axillary, inguinal and para-aortic lymph nodes were removed from the mice under sterile conditions. The lymph nodes were teased and the cells were enriched for T cells by passing the cells through Nylon wool columns. Recipient syngenic age and sex matched mice were gamma irradiated with 500 rads and were divided into four groups depending on the type of cells transferred i.v. Group 1 received T cell enriched lymph node cells from immunized donors (2x10^7 cells), Group 2 received T cell enriched lymph node cells from normal unimmunized mice (2x10^7 cells), Group 3 received
whole mono-nuclear cells from immunized donors (3x10^7 cells) and Group 4 received only saline and no cells (control group). Two hours after transfusion, all the four groups of animals were challenged with 1x10^7 live *M. tuberculosis* H37Rv given i.v. Four weeks after challenge, the animals were sacrificed and assessed for protection as described earlier. The results showed that adoptive transfer of T cell enriched lymph node cells conferred upon the recipient the ability to control progressive growth of *M. tuberculosis* H37Rv challenge as evidenced by significant reduction in the frequency of animals with gross pulmonary lesions as well as the number of viable units of *M. tuberculosis* H37Rv recovered from lungs, liver and spleen. The degree of protection conferred by T cell enriched lymph node cells from immunized donors was comparable to that given by direct M.w immunization.

5. To determine whether the immunity conferred by M.w was specific to *M. tuberculosis*, the ability of M.w immunized mice to clear *Listeria monocytogenes* infection was studied. Mice were immunized with heat-killed M.w s.c as mentioned earlier. Four weeks after immunization, both C3H (Bcg^T_1) and BALB/c (Bcg^S) strains of mice were infected with 1x10^4 *L. monocytogenes* i.v. At 1 hr, 18 hr and 72 hr intervals, six mice each were sacrificed and the number of CFUs of *L. monocytogenes* recovered from liver and spleen was determined. At the final interval
of study i.e. 72 hrs the normal growth of *Listeria* was not prevented in the liver of *M.w* immunized mice, thus resulting in bacterial recovery that was almost similar to that of normal unimmunized mice. A non-significant 3-fold reduction was observed in spleen. This trend was observed for BCG-susceptible BALB/c By J strain as well as BCG-resistant C3H/He NCrl strain thus demonstrating that *M.w* does not confer protection against heterologous non-specific challenge.

These experiments lead to the conclusion that *M.w* in heat-killed form is effective in conferring protection in mice and guinea pigs against challenge with live *M.tuberculosis* H37Rv. Both BCG-responder and BCG-nonresponder strains of mice were immunizable by *M.w*. Adoptive transfer experiments demonstrate the involvement of sensitised T cells in immunity. The resistance induced by immunization was specific for *M.tuberculosis* but not for *L.monocytogenes*. 