CHAPTER 2

REVIEW OF LITERATURE
2.1 Historical overview of tuberculosis

From times immemorial tuberculosis has ranked amongst the most feared and dreaded diseases that afflict mankind. The evangelist John Bunyon dubbed tuberculosis as 'the captain of all these men of death' and in India it was known as the 'King of Diseases'.

The turning point in the history of tuberculosis occurred in 1882, when Robert Koch described the isolation of the causative organism of the disease. Koch's studies also led to the development of the Old Tuberculin and the description of the necrotic hypersensitivity reaction termed the Koch Phenomenon in 1891. Furthermore, Old Tuberculin and the reaction it elicited, were used by Clemens Von Pirquet in 1907 to develop the tuberculin test, one of the most widely used and misunderstood of all the diagnostic tests.

The principles of vaccination were established by Pasteur, and many workers attempted to attenuate the tubercle bacillus for use as a vaccine. Edward Trudeau made a R1 strain, which was attenuated for guinea pigs, but no further development was undertaken.

A vaccine was eventually developed by Calmette and Guerin (Guerin, 1957) after passaging a supposedly bovine tubercle bacillus 230 times on potato slices soaked in bile and glycerol over a period of 13 years. This vaccine, Bacille Calmette Guerin (BCG), was first used in 1921 as an
oral vaccine for infants. Though, its routine use as a vaccine was delayed, by considerable controversy concerning its safety and by the Lubeck disaster in 1930 when many children got accidentally vaccinated with a virulent strain of Mycobacterium tuberculosis leading to 73 deaths, it was found to be one of the safest vaccines and is the most widely used vaccine till today. Extensive scientific studies in the recent decades have shown a variable protection conferred by it against pulmonary tuberculosis.

The first milestone in the treatment of mycobacterial diseases occurred in the mid twentieth century with the discovery of the first effective antitubercular drug. Streptomycin was discovered by Waksman in 1948 and the subsequent discoveries of isoniazid and other drugs at last removed the fear of tuberculosis. But the promises held out by drugs and BCG have probably done more to eradicate interest in the mycobacterial diseases than to eradicate the disease. The period from 1970 to the present time is of great fascination and excitement. Rifampicin was introduced and it made possible at last to contemplate short course curative chemotherapy for tuberculosis.

During the same time period, extensive studies were made on mycobacterial ecology, taxonomy, structural and biochemical studies, genetics and immunology. Although, these advances have broadened knowledge, they have contributed very little so far to the practical problems of the control of tuberculosis, such as early diagnosis and a
vaccine which could be an effective alternative to BCG.

Some workers are attempting to use 'genetic engineering' to develop more effective vaccines, while others are seeking better ways using the currently available BCG vaccine. It remains to be seen which approach proves most beneficial, but certainly the whole issue of vaccination against tuberculosis needs, and is receiving, a thorough reappraisal.

2.2 Magnitude of the disease

Tuberculosis is a major mycobacterial disease of great antiquity, which is prevalent world wide. About 30 million people are suffering from the disease and the annual deaths are about 3 million (Styblo, 1989). It is estimated that approximately 3-4 million new smear positive, another 3-4 million smear negative and, an additional extra pulmonary cases of tuberculosis may develop in World annually (Styblo, 1989). It continues to be a major problem in developing countries. In India 8 million people are reported to be suffering from the disease, with 500,000 deaths per year (ICMR Report, 1976).

2.3 Epidemiology

Tuberculosis continues to be a major public health problem in India. The prevalence of infection is of the order of about 40% in all age groups (as judged by positivity to PPD), rising to about 70% at age 35. Thereafter, it
remains almost constant. The incidence of infection is highest in individuals between the ages of 5 and 20 years. The risk of infection is of the order of 2-4 percent per annum (Park and Park, 1986). The prevalence of disease as determined by X-ray examination of the chest is of the order of 2% among total population aged 10 years and more, and of these about 20% (4 per 1,000 population) are open cases. The rough estimate of mortality from tuberculosis per 100,000 population is 60-80 (Barua, 1978).

2.4 Disease transmission

2.4.1 The causative organism

The disease is caused by Mycobacterium tuberculosis belonging to the genera Mycobacteria containing more than 50 recognised species. These bacilli are recognized by the development of rough, eugonic colonies with a characteristic buff tint, after 3-5 weeks of culture. M.tuberculosis reduces nitrate, produces niacin, loses catalase activity after heating at 68°C, and is usually sensitive to streptomycin, p-amino salicylic acid and isoniazid, unless derived from long treated "resistant" patients. If a strain of M.tuberculosis is isoniazid-resistant, it may be found to be catalase negative and may have reduced, or no virulence for guinea pigs. It is a strict mesophile, showing little or no growth below 30°C and above 39°C. This temperature range of growth, together with the failure of M.tuberculosis to grow on media containing 500 mg of p-nitrobenzoic acid per
one litre, enables this species to be distinguished from other slow growing non chromogens. \textit{M. tuberculosis H37Rv} is the virulent form of \textit{M. tuberculosis} while \textit{M. tuberculosis H37Ra} and R1Ra strain are avirulent forms of \textit{M. tuberculosis}.

### 2.4.2 Spread of infection

Tuberculosis is mainly contracted by the inhalation of mycobacteria via the respiratory route. Consequently, the initial lesion is in the lung from which organisms may be disseminated to other organs by the lymphatic or blood stream. Cornet (1889) and Chausse (1914, 1916) provided evidence that the chief source of infection was dust arising from dried sputum or dust liberated by brushing or shaking clothes contaminated by droplets expelled during coughing, speaking and sneezing by open cases of pulmonary tuberculosis, (sputum positive on direct smear examination) (Grzybowski and Allen, 1964; Rouillon \textit{et al.}, 1976). Sputum smear-negative patients, whether culture positive or not are classified as of very low infectivity.

The risk of infection is related to the number of bacilli in the aerosol, where only the fine particles containing one to three tubercle bacilli are infectious and remain suspended in the air stream that enters the alveolar spaces. The larger particles, containing bacillar clumps, which impinge on the mucous secretions in the nasopharynx and bronchial tree, are carried up by ciliary action of respiratory mucosa to the throat and are swallowed. Mucosal
surfaces are difficult to infect with tubercle bacilli. To do so, a large number of bacilli are required (Dannenberg, 1980). Disease caused by types of \textit{M.tuberculosis} other than the bovine type almost always begins in the lung as a result of inhalation of bacilli. The bovine type often enters the body by penetrating the tonsil or intestinal mucosa.

2.5 Spectrum of clinical presentation

Human tuberculosis is a disease of numerous manifestations, but there is an underlying pattern, which is briefly described here. Infection occurring in a person never previously exposed to \textit{M.tuberculosis} is termed primary tuberculosis. The primary infection consists of a lesion at the site of entry of the organism, which may be the lung, tonsil, intestine or, rarely, the skin. This lesion is termed the primary focus (Ghon, 1916), and together with involved regional lymph nodes is referred to as the primary complex or Ghon Complex (Ranke, 1928).

Hematogenous spread of the bacilli causes more widespread disease such as miliary tuberculosis, or infections of the central nervous system, genito-urinary tract, or bones and joints (Davies, 1971); it is particularly liable to cause fatal disease in children under three years of age.

Post-primary tuberculosis develops in the tuberculin-positive subjects, either as a result of exogenous re-
infection or by reactivation of organisms in a "healed" primary lesion. Large progressive cavitating lesions frequently develop in the apical lobes of the lungs and the walls of these cavities contain numerous bacilli. Some of these bacilli reach the lower parts of the lungs through the air passages by gravitation and contiguous spread and lead to the formation of secondary caseating lesions. Although, hematogenous and lymphatic spread is uncommon in post primary disease, than in primary infections, tuberculous ulcers caused by infected sputum may develop in the larynx and mouth and swallowed sputum may lead to similar ulcers in any part of the alimentary tract (Pagel et al., 1964). Post primary tuberculosis usually occurs five or more years after the primary infection (Styblo, 1978) and affects children as well as adults (Davies, 1971).

The term "open-tuberculosis" is applied to those cases in which bacilli are detectable in the sputum. This is often due to a cavity being in direct contact with a part of the bronchial tree so that bacilli gain access to the sputum.

2.6 Pathogenesis

The clinical manifestations and eventual outcome of tuberculosis, as of all infectious processes, are determined by the virulence of the pathogen and the nature of the host's immune response.
2.6.1 Virulence of *Mycobacterium tuberculosis*

Two different mechanisms seem to contribute to virulence viz. the synthesis of toxic substances, and the ability of the bacilli to survive within the phagocytic cells. The expression of virulence is affected by the occurrence of a specific immune response and by the non-specific innate resistance of the host to infection. Thus, in the rabbit, the bovine type of *M. tuberculosis* is much more virulent than the human type; the Asian type is of low virulence in the guinea pig and the virulence of the vole type is expressed only in voles, shrews and wood mice. Strains of the human or bovine type isolated from skin lesions are often less virulent for animals (Report, 1911), although passage through susceptible hosts causes some of them to acquire full virulence (Griffith, 1957). Likewise, bovine strains of *M. tuberculosis* isolated from horse are often of reduced virulence for rabbits and calves (Konyha and Kreier, 1971).

Several toxic substances have been isolated from tubercle bacilli; especially important are cord factor (Bloch, 1950) and sulpholipids (Middlebrook *et al.*, 1959). The structure and toxic properties of these compounds are discussed by Goren (1972), Ramakrishnan *et al.*, (1972), Goren and co-workers (1974a,b), Kato and Goren (1974), and Goren and Brennan (1979). The cord factor and other toxic glycolipids (Bloch, 1953; Noll *et al.*, 1956) inhibit the respiratory metabolism of the host cells by affecting
mitochondrial membrane function (Kato, 1968, 1969; Kato and ukushi, 1969; Kato and Tanaka, 1967). The sulpholipids can also inhibit the phagosome lysosome fusion within the macrophages, thereby enabling them to be virulent (Middlebrook et al., 1959; Goren, 1977). It is doubtful, if these lipids are directly associated with virulence (Grange et al., 1978). It has been shown that tubercle bacilli of phage type B contain very little sulpholipids, yet they are as virulent as strains of phage type A which contain large amounts of sulpholipids (Goren et al., 1982). Also, although South Indian strains contain only small amounts of sulpholipids; such small amounts are also found in a minority of fully virulent tubercle bacilli (Grange et al., 1978). However, sulpholipids bind to cord factor and the complex is highly toxic for mitochondria (Kato and Goren, 1974). There have also been studies on the biochemical parameters of virulence based on in vitro studies; these include reviews by Ramakrishnan et al. (1972), Kanai and Kondo (1974), Ratledge (1976, 1977) and Youmans (1979). Intermediary metabolism of virulent and avirulent forms of M. tuberculosis has been reviewed by Ramakrishnan et al., (1958, 1962, 1972).

2.6.3 Intracellular survival

Virulence of mycobacteria is almost certainly linked to the ability of the bacteria to survive within macrophages. How the alveolar macrophages kill the bacilli which they ingest is not exactly known. These phagocytes contain large
numbers of lysosomes (rich in digestive enzymes) (Cohn and Weiner, 1963; Dannenberg et al., 1963; Yarborough et al., 1967) and mitochondria (rich in oxidative enzymes) (Dannenberg et al., 1963; Oren et al., 1963). The various enzymes like lipases (Dannenberg and Bennett, 1963; Dannenberg et al., 1963; Braunsteiner et al., 1964; Elsbach, 1965; Hart and Payne, 1971), proteases (Dannenberg et al., 1963; Dannenberg, 1964), phospholipases (Franson et al., 1973; Franson and Waite, 1973) and lysozyme (Carson and Dannenberg, 1965) could hydrolyse bacterial cell walls by multiple enzyme action. Intracellular survival could partly be due to their ability to prevent fusion of the phagosomes with lysosomes (Lowrie et al., 1979). The inhibition of membrane fusion is presumably due to one or more mediators secreted by the bacilli: sulpholipids, polyglutamic acid, ammonium ions and adenosine monophosphate (AMP) being the possible candidates (Draper, 1981). There is also an association between virulence and resistance to hydrogen peroxide, which is one of the macrophage's defense mechanisms (Mitchison et al., 1963; Nair et al., 1964; Grange et al., 1977, 1978; Jacket et al., 1978). The recent evidence, although largely derived from work with mouse or guinea pig cells in vitro, strongly suggests that hydrogen peroxide is the major mycobactericidal agent produced by the macrophage (Lowrie, 1983). Strains highly resistant to isoniazid are attenuated and sensitive to hydrogen peroxide (H$_2$O$_2$) owing to the loss of peroxidase activity (Gayathri Devi et al., 1975).
2.6.5 Experimental animal models for studying pathogenesis and protection

The guinea pig model for tuberculosis has been used for assay of virulence of strains of *M. tuberculosis*, evaluation of antimicrobial agents and the pathogenesis of experimental airborne tuberculosis in immune and normal hosts. The understanding of the pathogenesis of tuberculosis has been enhanced through comparisons of the course of infection in vaccinated and non-vaccinated animals (Smith et al., 1970). Middlebrook (1952) developed a simple and inexpensive aerosol chamber that facilitated research on the pathogenesis of tuberculosis in laboratory animals under conditions that simulate human infection. The development of this chamber together with the discovery (Grover et al., 1967) that single cell suspensions of tubercle bacilli could be stored for decades at \(-70^\circ\text{C}\) without loss of viability, provided investigators with a reproducible and predictable method for infecting animals via the respiratory route under conditions that lead to the implantation and retention of as few as 1-2 colony forming units (CFU) of tubercle bacilli capable of replicating and producing primary lung lesions. Gardner (1922) described the resolution of experimental airborne tuberculosis induced in guinea pigs with low virulent R-1 strain of *M. tuberculosis*. He examined the course of infection in animals killed at intervals ranging from two weeks to 17 months after infection and demonstrated that caseous tubercles healed by resolution. Jensen et al.,
(1936) and Jensen and Bindslev (1937) challenged BCG vaccinated and non-vaccinated guinea pigs by the respiratory route in an aerosol chamber with a high exposure level, with as many as 200 nodules evident in a single section covering one lung lobe. Under these conditions, differences in number of bacilli revealed by Ziehl-Neelsen staining of tissue sections was evident as early as 8 days after infection. Middlebrook (1961) compared the number of virulent tubercle bacilli recovered from the lungs of BCG vaccinated and non-vaccinated guinea pigs killed one or seven days after infection via the respiratory route. The vaccination was done five days earlier by $1 \times 10^6$ CFU of BCG (strain Phipp) given subcutaneously (s.c.). The challenge level of approximately 5000 CFU/animal was used. Under these experimental conditions, an immune effect was evident as early as one week after infection, demonstrated by significantly more organisms recovered from non-vaccinated as compared to vaccinated guinea pigs. Smith et al., (1970) contrasted the course of infection in BCG vaccinated and non-vaccinated guinea pigs infected via the respiratory route under conditions that led to the development of three primary lesions on the surface of lung lobes. Groups of vaccinated and non-vaccinated animals were killed at three day intervals and the number of bacilli that could be recovered from the lungs was determined. Their data showed that despite the presence, at the time of challenge, of circulating sensitised T lymphocytes (evident from skin test data), replication of tubercle bacilli in immunized animals was the same as in non-
immunized animals until the number of bacilli in each primary lesion reached $1 \times 10^3$ CFU. Although, the number of primary lesions did not differ significantly for the two groups, hematoxylin and eosin staining of primary lesions 24 days after infection revealed major differences in the size of the lesions and in the extent of caseous necrosis. Other studies, by the same group, have shown that transport of bacilli to the spleen and to those lung lobes that did not have a primary lung lesion was significantly delayed in onset and was reduced by a 100 fold in vaccinated animals (Harding and Smith, 1977). Another important observation made by Smith’s group was that guinea pigs infected by the respiratory route undergo a naturally occurring bacilleemia (Smith, 1970; Smith and Weigeshaus, 1989). This observation is of special interest because of the importance of bacillary phase of infection in the pathogenesis of tuberculosis in humans. The bacilleemia occurs in non-vaccinated guinea pigs about the third week of infection and can be detected by recovery of the organisms from the spleen. Cohn (1969) studied the temporal change in the number of tubercle bacilli recovered from excised primary lung lesions of guinea pigs infected with approximately 150 CFUs of \textit{M. tuberculosis} H37Rv by the respiratory route. The bacillary population in the lesion reached $4-5 \log_{10}$ by the 14th day and then reached a stationary population of about $5.0 \log_{10}$ from the 16th to 24th day. Ho et al. (1978) studied the pathogenesis of tuberculosis in non-vaccinated guinea pigs
infected via the respiratory route with approximately 2 virulent organisms capable of multiplying and initiating primary lesions. At intervals ranging from 16-56 days after infection, animals were killed to determine the number of bacilli recoverable from metastatic foci in primary lesion free lung lobes seeded as a result of bacillemia. The results indicated that bacilli continued to multiply in metastatic foci even though infection-immunity had led to the termination of log-phase multiplication in the primary lesion. These results suggested that the factor(s) responsible for control of multiplication in the primary lesions were not systemically distributed. The pathogenesis of experimental airborne tuberculosis was compared in guinea pigs infected with virulent (H37Rv) and attenuated (H37Ra) \textit{M. tuberculosis}. Whereas the course of infection with the virulent strain was as described above, the attenuated strain multiplied 10,000 fold during the first three weeks and then continued on to a self limited process with gradual elimination of the organisms (Alsaadi and Smith, 1973). Other contributions of animal models to the understanding of the pathogenesis of tuberculosis can be briefly described as follows: (1) Lurie's (1964) classical studies of tuberculosis in inbred strains of rabbits that differed in their susceptibility to tuberculosis and his early studies in rabbit eye chambers, demonstrated the role of macrophages in the immune control of mycobacteria; (2) Dannenberg's animal model studies elucidated the fundamental events in the development of delayed type hypersensitivity (DTH) and
immunity (Courtade et al., 1975); (3) the work of Riley et al. (1959) in which guinea pigs were used for monitoring the exhaust air from patients' rooms in a hospital as a means of quantitating the number of droplet nuclei per cubic meter of air generated by a tuberculosis patient and (4) Mitchison's (1964) demonstration that 60-70% of fresh isolates of tubercle bacilli showed a reduced ability to produce gross tuberculosis in guinea pigs.

2.7 Immunology of tuberculosis

Mycobacteria, in common with other intracellular parasites owe their virulence to their ability to survive within the macrophages. Protective immune reactions in mycobacterial disease are of the so-called cell-mediated type and serve to enhance the ability of the macrophages to inhibit or destroy the invaders. Humoral immune response, i.e. antibody production certainly does occur, but there is no evidence that they play any major role in host defense (Grange, 1988). Most individuals exposed to or infected with \textit{M.tuberculosis} produce antibodies against this bacillus. This serological response has been known for several decades although its role either in the pathogenesis of the disease or protective immunity is poorly understood.

2.7.1 Cell Mediated Immune Response

It is generally believed that acquired resistance to mycobacteria is a cell-mediated process that starts when
sensitised thymus dependent (T) lymphocytes recognise bacterial antigens on antigen presenting cells. Antibodies alone seem to contribute little to resistance (Reggiardo and Middlebrook, 1974) and activated macrophages appear to be the main effector cells (North, 1974; Walker and Lowrie, 1981). Acquired cell mediated immunity is known to involve the generation, within the host, of protective T lymphocytes (Lefford, 1975; North, 1973; Orme and Collins, 1983). Immunological phenomenon seen in mycobacterial diseases consists, as in other infections, of recognition, response and reaction.

Antigen recognition is a property of the lymphocytes which are divided into two main subsets; the B cells and T cells. The former mature into antibody secreting plasma cells, while the latter are involved in the initial recognition of the antigen, the regulation of the immune response, cell mediated cytotoxicity and the secretion of hormone like molecules that affect other cells of the immune system. All mature T lymphocytes express the CD2 and CD3 determinant (CD = Clusters of differentiation antigens) and the subsets of T cells possess other unique CD antigens in addition to these "universal" T cell markers, these are 57-KDa glycoprotein called CD4 which is expressed on T cells of 'helper' variety and 32 KDa glycoprotein called CD8 present on T cells belonging to the 'suppressor' and 'cytotoxic' subsets.

It is generally believed that the functional property
of a given T cell could be inferred from its phenotypic surface markers (Reinhertz and Scholossma, 1980). This concept is no longer valid, because dissociation between classic cell markers and functions are known to occur. For example, although most cytotoxic T cells are CD4⁻, clones of CD4⁺ cytotoxic T cells are known to exist (Krensky et al., 1982).

The inhaled mycobacteria are initially engulfed by polymorphonuclear lymphocytes (PMN) but are rapidly released. In contrast to their behaviour in macrophages, the bacilli do not inhibit the fusion of lysosomes with phagosomes in PMN; nevertheless, PMN are unable to destroy them (Smith et al., 1979a).

After release, they are lodged in the periphery of the lung and are engulfed by alveolar macrophages. Some bacilli are transported with macrophages to the regional lymph nodes, where antigen is processed and presented to lymphocytes, which undergo clonal expansion. In the meantime, the bacilli at the initial site of infection and in the lymph nodes proliferate and some are carried further to the lymphatics and blood stream.

Within 24 hours, the bacteria are phagocytosed by macrophages, which, by that time, are the predominant cells in the lesions (Adams, 1975; Closs and Haugan, 1975). The alveolar macrophages appear to play only a limited defensive containment role against M.tuberculosis. Even in sensitised
subjects it is not the alveolar macrophages but the recruited blood borne macrophages that destroy the organisms (Lefford, 1980).

When the immune response has developed, activated macrophages and lymphocytes cluster around the invading mycobacteria. In the presence of lymphokines, the activated macrophages mature into the so-called epithelioid cells and some of them fuse to form giant cells. This forms the characteristic lesion of chronic infection known as the granuloma, at the site of mycobacterial growth. These granulomas consist of epithelioid cells, multinucleated giant cells, immigrant blood monocytes, and CD4$^+$ as well as CD8$^+$ T cells (Beck et al., 1986, Kaplan et al., 1986). It appears that a proportion of the mononuclear phagocytes within the granuloma contain intracellular bacilli that they have not been able to kill. Although these host cells contribute to protection by containing the pathogen to circumscribed foci and thus preventing them from colonizing other sites of the body, they are not sufficient for bacterial clearance. Lysis of these cells seems to be an important step, since in a well organised, productive granuloma, released bacilli can be taken up by fresh blood monocytes ready to kill the organisms after appropriate lymphokine activation. Furthermore, bacteria released into the center of the granuloma might succumb to the hypoxic environment. As long as the lesions caused by cytolyltic T cells and activated macrophages remain small, they can be tolerated by the host. Excessive tissue
destruction by these mechanisms, however, could result in caseation and liquefaction of the granuloma, followed by bacterial dissemination. Hence, detrimental consequences for the host will predominate under these circumstances. These detrimental consequences could be immuno-suppression and delayed hypersensitivity.

According to classical dogma, helper T cells capable of activating increased antimicrobial capacities in macrophages play a major role in host defense against intracellular bacteria, including \textit{M.\textit{tuberculosis}}, whereas cytolytic T cells that lyse infected target cells are important in combating viral infections (Zinkernagel and Doherty, 1979). Recently Kaufmann's group (1979, 1985) has concluded from their studies on T cell lines and clones of either L3T4 or the Lyt 2 phenotype that both L3T4\(^+\) (CD4\(^+\)) and Lyt2\(^+\) (CD8\(^+\)) T cells participate in the immune response against \textit{M.\textit{tuberculosis}}. Kaufmann (1989) has presented evidence that both CD4\(^+\) and CD8\(^+\) T cells are generated in experimental tuberculosis. In their study, mice were thymectomised to prevent regeneration of new T cells and then treated twice with monoclonal antibodies to the CD4 or CD8 molecule which are capable of selectively depleting the CD4 or CD8 positive T cell subset respectively (Cobbold \textit{et al.}, 1984). These mice were then infected with \textit{M.\textit{tuberculosis}} and the number of viable organisms in spleens was determined 3 weeks later (Muller \textit{et al.}, 1987). Depletion of either T cell subset resulted in a significant increase of the bacterial load, with CD4\(^+\)
T cell-depleted mice showing more severe effects. Concomitant depletion of both subsets had no worse an effect than depletion of CD4\(^+\) T cells alone. Earlier, Orme and Collins (1984, 1987) in the tuberculosis system and Kaufmann et al., (1979) in the listeriosis model suggested that both CD4\(^+\) and CD8\(^+\) T lymphocytes are required for the acquisition of resistance against tuberculosis.

The possible role of gamma-delta T cells in primary response to \textit{M.tuberculosis} was studied by Janis et al., (1989) in the murine system. They found that after priming with \textit{M.tuberculosis}, there was 20-25 fold increase in the absolute number of CD3\(^+\) CD4\(^-\) CD8\(^-\) V\(\beta\) \(8\) population in the draining lymph nodes of mice immunized with \textit{M.tuberculosis} as compared to unprimed lymph nodes. The effect was observed 7-9 days after immunization. The gamma-delta T cells appear to be roughly ten times as active as the alpha-beta T cell subset in primary immune response to \textit{M.tuberculosis}. These cells are important in generating lymphokines necessary for initiating immune response (IR) to \textit{M.tuberculosis}. T cells bearing gamma-delta T cell receptor (TCR) responded to solubilised \textit{M.tuberculosis} antigens in vitro but this response did not require major histocompatibility complex Class II recognition.

Kaufmann (1989) has also shown that CD4\(^+\) T cells and some CD8\(^+\) T cells produce IFN-\(\gamma\) (interferon gamma) after appropriate stimulation and that IFN-\(\gamma\) (either T cell
derived or recombinant) induces strong tuberculostatic or tuberculocidal activities. These findings strongly support the notion that macrophage activation by lymphokines represents a significant mechanism of protection against tuberculosis. By means of an autocrine positive feed back mechanism, vitamin D has been reported to enhance the antimycobacterial effect of IFN-γ (Rook, 1986). It has also been shown that macrophages activated by IFN-γ have a limited antimycobacterial effect. Although, IFN-γ is an important macrophage activator, but it is not the only one. Experiments in human system indicate that factors derived from \textit{M. tuberculosis} reactive T cells, which are distinct from IFN-γ, can activate macrophages (Andrew, 1984). In the human system, IFN-γ activated blood monocytes have been shown to enhance replication of macrophage associated mycobacteria, which is in contrast to the studies in the murine model (Douvas, 1985).

In addition to its microbicidal activity, the macrophage synthesises and secretes many important compounds that affect the pathogenesis of mycobacterial disease. These include some of the acute phase reactant proteins, vasoactive peptides, and proteases that liquefy necrotic tissues and contribute towards the formation of the tuberculous cavity. In addition, macrophages secrete a substance known as cachectin which is thought to be responsible for the extreme wasting associated with advanced tuberculosis. This substance is identical, or extremely similar, to tumor
necrosis factor (TNF) (Beutler et al., 1985) which may contribute to the necrotic reactions seen in delayed hypersensitivity.

2.7.2 DTH responses

After primary infection, there is a period of bacillary multiplication before clinical disease develops. The first manifestation is a conversion to tuberculin positivity, which may be accompanied by fever, less often by erythema nodosum, and rarely by phlyctenular conjunctivitis (Wallgren, 1948). The mechanism of tuberculin reaction and its relationship to protective cell-mediated immune reactions is the subject of a long and unresolved controversy.

Koch (1891), observed that an intradermal injection of virulent *M. tuberculosis* led to a progressive systemic infection in the guinea pig. A further intradermal injection of bacilli during the infection led to a local necrotic reaction with destruction and shedding of the organisms. The elicitation of the so-called Koch phenomenon had no effect on the progress of the systemic disease. Nevertheless, it became generally accepted that DTH was of benefit to the host - a view that is still prevalent. Romer (1908) observed that tuberculin hypersensitivity in animals was associated with increased resistance to reinfection, provided that the hypersensitivity reaction was not too intense. Other early workers (Austrian 1913a, 1913b; Krause and Willis, 1919) showed that the induction of tuberculin hypersensitivity in
guinea pigs reduced their resistance to subsequent challenge with *M. tuberculosis*. Wilson and his colleagues (1940) observed lack of correlation between cell mediated immunity (CMI) and DTH in guinea pigs. Although, a moderate degree of DTH was associated with protection, animals with a high degree were less protected than those with none.

These findings were supported by the clinical observations of Turner (1953), who found that quiescent tuberculosis was more likely to be reactivated in patients with either a negative or a strongly positive tuberculin reaction than in those showing a moderate response.

Youmans (1979) has cited several studies in support of the concept that CMI and DTH are distinct and dissociable phenomena and, in particular has drawn attention to the fact that primary tuberculosis lesions are often resolving by the time tuberculin conversion occurs, strongly suggesting that CMI is already active.

Rook and Stanford (1979) suggested that the controversy surrounding the protective role of DTH has arisen because this term is applied indiscriminately to more than one phenomenon. They demonstrated that mycobacterial infections lead to the development of three types of 'delayed' skin-test reactions: the poorly understood reaction of Jones and Mote (1934), an early developing non-necrotic reaction corresponding to the macrophage activation described by Mackaness (1967), and a late developing necrotic reaction
corresponding to the Koch phenomenon. In mice the more pathogenic mycobacteria either suppress or fail to elicit the non-necrotic reaction which, being associated with macrophage activation, is appropriate for the control of systemic disease. The necrotic reaction, on the other hand, appears to be more beneficial when the infection is restricted to the skin. It is relevant that of the several strains of mice studied, the C57BL strain is the most resistant to subcutaneous challenge with \textit{M.tuberculosis} but the most susceptible to intravenous challenge. The role of CMI in tuberculosis has been extensively reviewed by Lagrange (1984).

\subsection*{2.7.3 The immune spectrum of disease manifestation}

As in leprosy, existence of a spectrum of immune response has been postulated in tuberculosis (Lenzini \textit{et al.}, 1977). Reactive cases, showing a good cell-mediated immune response, comparatively few bacteria and a good response to treatment, at one end, and unreactive cases, resembling lepromatous leprosy in showing numerous organisms associated with absence of a cell-mediated immune response, were described. Bhatnagar and his colleagues (1977) also described a spectrum of immune response in tuberculosis with self-limiting subclinical cases at one end and miliary tuberculosis at the other end. Proudfoot (1971), however, showed that miliary disease was accompanied by an immune response leading to the formation of millet seed like granulomas. In cryptic disseminated disease, necrotic foci
teem with organisms, but there is little or no cellular reaction. The latter disease, which is often rapidly fatal, is sometimes referred to as 'diffuse' in contrast to 'nodular' tuberculosis, or as the Yersin type of disease on account of its histological similarity to Yersinia pseudotuberculosis (Y.pseudotuberculosis) infection in the guinea pig (Skinsnes, 1968).

There appears to be no direct parallel between the spectra of immune response in tuberculosis and leprosy (Rook and Stanford, 1979; Kardjito and Grange, 1980). In tuberculosis, activation of the macrophages containing the bacilli is advantageous, whereas, in leprosy a cytotoxic response, releasing bacilli from cells which cannot be activated, is desirable.

2.8 Diagnosis of tuberculosis

Definitive diagnosis is achieved by culture of the organisms but the procedure being slow and resource intensive, is not routinely followed in most of the developing countries. Also, sputum smear examination for acid fast bacilli in pulmonary tuberculosis may give a less than 50% positive yield (Blair et al., 1976; Kim et al., 1984). Sensitivity of smear examination is even lower in extrapulmonary tuberculosis (Daniel, 1987). Moreover, the property of acid fast staining is not exclusive to M.tuberculosis. Other available methods for diagnosis are also beset by number of limitations and problems. Tuberculin
skin test, measuring delayed type cutaneous hypersensitivity is commonly employed as an aid in diagnosis but this test too is subject to false positive and false negative reactions and to subjective differences in application and interpretation. A further complication is reflected in the possibility that an existing delayed type hypersensitive response can be augmented during serial skin testing of patient populations (Glassroth et al., 1980). Its incapability in differentiating patients with active and inactive tuberculous disease from each other and from healthy, tuberculin-positive and tuberculin-negative persons has also been identified (MacLean, 1975; Glassroth et al., 1980).

Efforts to develop serological assays based mainly on antibody detection have faced with problems of both sensitivity and specificity. Among the better and more sensitive methods are radioimmunoassays (RIA), enzyme immunoassays (EIA) and the modified soluble antigen fluorescent antibody (SAFA) test. RIA is highly sensitive but has several drawbacks: short shelf life of reagents, problems of waste disposal, requirement of costly equipment for counting radioactivity and radiation hazards. SAFA is sensitive but requires the costly spectrophotometer. The EIAs for sero-diagnosis of tuberculosis have been extensively reviewed by Daniel and Debanne (1987). Antigen preparations that were utilised in most of these studies contained moieties that were common to many bacterial species leading to serological cross reactivity (Bardana et al., 1973;
Mitchison et al., 1977; Chaparas et al., 1980; Winters and Cox, 1981; Grange, 1984). The assays based on PPD (Kalish, 1983), antigen 5 (Balestrino et al., 1984; Ma et al., 1986) and crude culture filtrate of M. tuberculosis (Nassau et al., 1976) have been recently reviewed by Daniel (1989). Detection of immune complexes in serum of patients has been suggested as potential diagnostic test (Bhattacharya et al., 1986) but here again the sensitivity appears to be low (Johnson et al., 1981).

Different mouse monoclonal antibodies to defined antigenic determinants of M. tuberculosis have been employed in various kinds of immunoassays to detect antibodies or antigen in patient samples. Ivanyi et al., (1983) have reported a serum antibody competition test (SACT) based on the use of 125 I-labelled monoclonal antibody (TB-72) directed against M. tuberculosis. TB-72 alone could detect 63\% of untreated active pulmonary tuberculosis patients in a U.K. study (Hewitt et al., 1982) and 83\% in Indonesian patients (Hoeppner et al., 1987). The combined score of the triplet of antibodies (TB72 + TB68 + TB23) increased the positive serology of London patients to 71\% and produced as much as 95\% of positive detection in Indonesian patients.

If there were immunodominant epitopes on the antigen other than those defined by tested monoclonal antibodies, an improved rate of detection might be achieved using purified whole molecules to measure antibody levels. Moreover, the
SACT, being a radioimmunoassay is unlikely to be favoured as routine diagnostic test in developing countries, where in fact the disease is much serious a problem, owing to its inherent disadvantages in terms of initial high costs of installation and safety considerations. A monoclonal antibody binding to the surface antigen of \textit{M. tuberculosis} and having restricted cross-reactivity was developed in our Institute (Kapoor et al., 1990). It reacts with pathogenic strains of \textit{M. tuberculosis} (H37Ra) and different strains of \textit{M. bovis} BCG. Preliminary studies employing the competitive enzyme immunoassay have shown the presence of antibodies to epitope recognised by P6 in sera of pulmonary tuberculosis patients in Northern India. BCG vaccinated apparently healthy individuals did not have antibodies to this epitope. Its use in the immunodiagnosis of pulmonary as well as extrapulmonary tuberculosis seems to be very promising (H.V. Batra, personal communication).

To overcome the limitations of SACT, a simple direct binding ELISA was developed by Jackett et al. (1988) using purified antigen molecules. Antigens utilized were 14-, 19-, and 38-kilodalton (kDa) protein antigens of tubercle bacilli obtained by monoclonal antibody affinity column chromatography, 65-kDa antigen from \textit{M. bovis} BCG, purified from overproducing recombinant \textit{Escherichia coli} K-12 cells (Thole et al., 1987), and purified lipoarabinomannan (LAM) from \textit{M. tuberculosis}. For smear-positive samples, the best sensitivity (83\%) was achieved by exclusive use of the 38-kDa
antigen or its corresponding monoclonal antibodies. For smear-negative samples, levels of antibodies binding to the 19-kDa antigen showed a lower sensitivity of 62% compared with the control group and 38% compared with the contact group. Titers of antibody binding to the 14-kDa antigen were raised in *M. bovis* BCG-vaccinated contacts, indicating that the greatest potential of this antigen may be in the detection of infection in a population for which tuberculin testing is unreliable.

Recent approaches applicable to the recognition of mycobacteria in clinical specimens are: "antigen detection immunoassays" and "use of nucleic acid probes" for the recognition of specific DNA or RNA base sequences. Strauss and Wu (1980) established a RIA for mycobacterial antigens that uses rabbit antiserum to tuberculin PPD. This assay has been used in two clinical studies (Strauss and Wu, 1980; Strauss et al., 1981). Another group (Kadival, 1982) established a RIA for mycobacterial antigens using a commercially available rabbit antibody to Bacille Calmette Guerin (BCG).

Originally developed as tools for molecular biology and genetic engineering research, DNA probes are specifically designed for detecting and identifying specific nucleotide sequences. DNA probes for detection of *Mycobacterium tuberculosis* have been developed by several groups. While Shoemaker et al. (1985) and Roberts et al. (1987) have shown the usefulness of whole chromosomal DNA as probes, a series of
reports appeared where complementary DNA to conserved ribosomal RNA (Paterson et al., 1989) or cloned genomic fragments (Pao et al., 1988) or highly repetitive DNA sequences (Reddi et al., 1988; Eisenbach et al., 1988) have been shown to be M.tuberculosis complex specific, with a sensitivity of 100 pg which is equivalent to $10^4$ bacilli.

The detection limit of DNA probes has been further refined by in vitro amplification of target DNA using the polymerase chain reaction (Rubina et al., 1990; Shankar et al., 1990), but the feasibility of such an approach in field conditions for epidemiologic studies remains doubtful.

2.9 Genetic factors in tuberculosis

Tuberculosis often runs in families but to what extent this is due to genetic factors, or exposure to a source case within the household is difficult to determine. Most lines of evidence supporting the role of genetics in the pathogenesis of tuberculosis and related infections have been indirect. These indirect evidences were racial differences in susceptibility (Motulsky, 1960), similar susceptibility in twins (Comstock, 1978) and cosegregation of known chromosomal markers with the disease, both in families and in outbred populations (Singh et al., 1983).

The precise role of genetic factors in the variable susceptibility to mycobacterial infections in human populations has not been clearly established. This is due to
the fact that related family members share not only their genetic pool but also the air they breathe and the food they eat. Also, host response to any infectious agent cannot be expected to be regulated solely by a single gene. The possession of a particular allele at one genetic locus may dramatically influence the course of disease in certain individuals. At the level of whole population, many genes may be expected to be responsible for the disease profile, which is frequently spectral in mycobacterial infections (Lenzini et al., 1977). Furthermore, many interrelated processes that manifest themselves as susceptibility or resistance to the acquisition of infection, susceptibility or resistance to the establishment of infection, progress or clearance of infection, or susceptibility and resistance to reinfection take place within the host exposed to an infectious agent.

2.9.1 HLA Studies in human tuberculosis

Several studies have been carried out to study the genetic basis of susceptibility to tuberculosis. Associations have been reported between HLA class-I as well as class-II alleles and tuberculosis. While Singh et al., (1983) found DR2 to be associated with susceptibility to pulmonary tuberculosis, Paizieu (1986) found B12, besides DR2, to be more frequent in the tuberculous patients as compared to healthy persons. Khomenco et al., (1981) have found an increased occurrence of Bw49 antigen while Zervas et al., (1987) observed a significantly increased frequency
of the short HLA-B27 antigen in Greek adult patients suffering from pulmonary tuberculosis. Xu et al. (1986), found increased frequency of the HLA-A11 and B15 antigens and a decreased frequency of the HLA-Cw3 antigen in Chinese patients while Hafez et al., (1985) found no association between HLA and susceptibility to tuberculosis in Hong Kong Chinese. However, they found significantly increased frequencies of A2 and B5 in Egyptian population. In another study (Chandanayingyong et al., 1988), an increase in the frequency of HLA-Bw46 and DR4, and a decrease in the frequency of HLA-B12 were found when compared with the matched controls. Their findings suggested that HLA-B12 confers resistance and HLA-Bw46 and DR4 are associated with susceptibility.

Teren et al., (1985), showed a decrease in HLA-A3, A9, B5, B8, B13, B17 and B27 antigens, while Cox et al., (1988) found HLA-DR3 to be significantly decreased in patients with tuberculosis.

Since all these studies have been done in different populations and the results differ from population to population, no specific conclusions can be drawn from these studies.

2.9.2 Role of non-HLA regions in tuberculosis

It is likely that non-HLA regions also control the immune response. A single autosomal dominant gene, Bcg,
controls the susceptibility of mice to the establishment of infection after exposure to mycobacteria. In the mouse, this gene has been mapped to the centromeric region of chromosome 1 within a linkage group which shows structural homology with a conserved region of human chromosome 2 (Skamene, 1989).

It is now well established that various inbred strains of mice differ widely in their ability to control the proliferation of intravenously administered mycobacteria. For example, it was found that early resistance to infection with Mycobacterium bovis (Strain BCG Montreal) as well as to a variety of other BCG substrains is under the control of a single dominant gene designated Bcg that exists in two allelic forms in mouse, BcgR (resistant) and BcgS (susceptible) (Forget et al., 1981; Gros et al., 1981). The strain distribution pattern of resistant (BcgR) and susceptible (BcgS) alleles was found to be very similar to that of two isoenzyme loci, IDH-1 (isocitrate dehydrogenase) and Pep-3 (dipeptidase 3) located on the centromeric part of chromosome 1. The gene order was established by recombination frequencies to be IDH-1, Bcg and then Pep-3 (Skamene et al., 1982). BcgS (C57BL/6, B10A, BALB/c) mice allow progressive multiplication of BCG in the reticuloendothelial system (RES) during the first three weeks of infection, associated with severe granulomatous hepatitis. In contrast, only a few granulomas were found in the liver of BCG-resistant (A/J, C3H/HeN and CBA/N) strains of mice,
and there was no significant growth of mycobacterium in RES. (Pelletier et al., 1982; Buschman et al., 1986). The presence of a large number of granulomas in susceptible mice most likely reflects the expression of a cellular immune response to BCG (Adams, 1976; Boros, 1978) in those animals, because the kinetics of its appearance resembled the development of other immunologic phenomena followed in their study like splenomegaly and development of DTH. The development of cell-mediated immunity resulted in the acquired resistance of BcgS mice to a later challenge with homologous (BCG) and heterologous pathogens. The genetically resistant host obviates the need for generation of acquired mechanisms of immunity. Such a host develops nil or low levels of secondary specific anti-BCG or non-specific immunity (Pelletier, 1982).

The identification of the Bcg gene and of the in vivo trait of resistance to infection that is under its control, permitted the development of mouse strains that are Bcg congenic, i.e. genetically identical except for the small chromosomal segment containing the Bcg linkage group (Potter et al., 1983). The functional studies analysing the cellular mechanisms controlled by this gene could, therefore, be performed in a system in which interference from the remainder of the genome was almost non-existent. This genetic locus, in addition to regulating innate resistance to mycobacterial infection, controls early host response to Salmonella typhimurium and Leishmania donovani (Plant and
Glynn, 1982; Bradley, 1979). Infection with *M. lepraemurium* has also been shown to follow Bcg gene distribution (Brown et al., 1982; Brown and Glynn, 1987). As all the three pathogens restricted by the Bcg gene are intracellular parasites, it is quite possible that the gene controls some facet of macrophage function (Skamene et al., 1982). The depletion of T-cell, B-cell and the natural killer (NK) cell populations from the resistant animals not only did not alter their response but the animals remained resistant even after total body irradiation with 900 R (Denis et al., 1988).

Further, it was found that the trait of resistance to these infections could be transferred by bone marrow cells into radiation chimeras, thus indicating that this trait was expressed by the progeny of hemopoietic precursor cells (Orme et al., 1986). The only manipulation that transformed the genetically resistant mouse strain into a phenotypically susceptible strain was chronic treatment with silica (Gros et al., 1983). Thus, by exclusion, mature macrophages remained the only candidates for the cell population that expresses the Bcg gene. It has been confirmed, both in vivo and in vitro, that the cell that expresses the Bcg gene is the resident macrophage (Gros et al., 1983; Stach et al., 1984).

Various studies have been done in order to look into the exact facet of macrophage function that is regulated by Bcg gene. It was shown that Bcg<sup>T</sup> macrophages, isolated from the peritoneal cavity or the spleen of normal, uninfected
donors were superior to \textit{Bcg}\textsuperscript{S} macrophages in their ability to interfere with the intracellular growth of BCG (Stach \textit{et al.}, 1984).

A recent study by Johnson and Zwilling (1985) has shown that activated peritoneal macrophage from BCG resistant mice expressed Ia continuously after \textit{in vitro} culture, whereas macrophages of BCG susceptible mice expressed Ia transiently. These authors suggested that the phenotypic expression of the \textit{Bcg} gene \textit{in vivo} is directly related to a superior macrophage antigen presentation by \textit{Bcg}\textsuperscript{R} macrophages. The \textit{Bcg}\textsuperscript{R} and \textit{Bcg}\textsuperscript{S} macrophages differ in their state of activation: the \textit{Bcg}\textsuperscript{R} macrophages are already primed for activation. The \textit{Bcg}\textsuperscript{R} macrophage, when exposed to the membrane perturbation caused by the phagocytosis of mycobacteria, becomes promptly activated for anti-mycobacterial activity. By contrast, \textit{Bcg}\textsuperscript{S} macrophage requires a sequence of priming and activating stimuli (including mycobacterial cell wall products and lymphokines) before the full extent of its anti-mycobacterial activity is exposed. However, it still remains a matter of controversy as to whether this phenomenon is the responsible factor for the early resistance of \textit{Bcg}\textsuperscript{R} mice to BCG \textit{in vivo} or if it represents but one facet of a pleiotropic effect of the \textit{Bcg} gene. That antibacterial activity against a wide range of pathogens is superior in \textit{Bcg}\textsuperscript{R} macrophages, is shown by Lissner \textit{et al.}, (1985) who demonstrated superior bactericidal activity of genetically resistant macrophages against a diverse group of intracellular and extracellular
bacteria. Resistance in vivo to many of these bacteria (*Salmonella typhi*, *E.coli*, *Staphylococcus aureus*, *Corynebacterium diphtheriae*) is not solely restricted by the *Bcg* gene, implying that mechanisms other than direct macrophage-pathogen interaction operate in the complex host defense systems. A similar argument can be used to explain the fact that resistance of inbred mice to *B. abortus*, a microorganism whose antigen is also presented more effectively by *Bcg* macophages, does not follow the *Bcg* gene pattern in vivo (Le Ganec et al., 1975).

2.10 Animal models for tuberculosis

2.10.1 Monkeys

The higher apes are highly susceptible to tuberculosis. Non-human primates constitute the obvious experimental animal for testing protective efficacy of antituberculosis vaccines. But their increased handling problems, costs and reduced availability tend to limit their more widespread use in this regard. Histopathological studies of aerogenically challenged Rhesus monkeys indicate that the disease pattern and resulting immune responses are both very similar to those seen in man and it is likely that further studies with this host species will be attempted in future (Barclay, 1970; Barclay et al., 1973; Good, 1968). Good (1968) described experiments where virulent tubercle bacilli were instilled into the bronchi of monkeys at a dose of 200 to 500 organisms per animal. The course of infection
was reasonably consistent and non-immune monkeys developed extensive pulmonary tuberculosis with caseation and necrosis. Prior immunization with BCG modified the course of disease and induced tuberculin hypersensitivity. In immunized monkeys, the extent of lung involvement was more limited, although some monkeys still developed numerous lesions.

2.10.2 Rabbits

The earliest experimental studies of the spread of tuberculosis disease were carried out in this host species (Baldwin, 1910). The rabbit is a particularly useful experimental animal in this regard since it develops cavitary lung disease following aerogenic challenge and can directly infect normal animals housed in close proximity. For this reason, tuberculous rabbits may pose special health hazards for laboratory workers. Most studies have been carried out with randomly bred rabbits and this has been a limiting factor with respect to adoptive transfer studies which normally require highly inbred stock. Care has to be exercised in the selection of rabbit stocks for such experiments, since this host is extremely susceptible to Pasteurella multocida infection (snuffles) which can result in chronic lung infections (Collins, 1977). Rabbits are also extremely susceptible to Y. pseudotuberculosis infection which may confuse the issue. Because of their size and expense, especially when involving long term infection studies, very little work is currently being carried out in rabbits.
2.10.3 Guinea pigs

Guinea pigs are exquisitely susceptible to experimental infection with M. tuberculosis. They are commonly infected experimentally by the intramuscular (Mitchison et al., 1961), subcutaneous (Feldman, 1943; Krause, 1920) or respiratory route (Riley et al., 1959; Smith et al., 1979b) and less commonly by the intravenous route (Widstrom, 1946; Karlson, 1959; Karlson and Feldman, 1953; Wallace et al., 1961). With the exception of those using respiratory challenge, most investigators challenge animals with an inoculum containing the equivalent of $1 \times 10^5$ to $1 \times 10^8$ CFU. With respiratory challenge, significantly lower challenge levels are ordinarily used, i.e. in the range of 1 to 100 CFU.

The major advantage of the guinea pig as an animal model of tuberculosis is that the CMI responsiveness of this species is a closer approximation to that of humans than any other common laboratory animal. The histopathology of tuberculosis in guinea pig and man is closely similar, in that caseous granulomas containing epithelioid and Langhan's giant cells are produced in both species. These two species also develop similar tuberculin DTH reactions of an indurated type, in which edema is relatively inconspicuous, andcellularity is dense.

2.10.4 Mice

Most rodents are not naturally susceptible to M. tuberculosis infection. However, progressive disease can
be induced if the mouse is infected with a relatively large
dose (1x10^6 to 1x10^8 viable units) of virulent M.tuberculosis
(Gerstly and Thomas, 1941; Donovick, 1949). Outbred Swiss
mice have been extensively used in experimental tuberculosis
studies for over 50 years (Kanai, 1967). However most of the
early studies were limited to survival determinations only
(Wiess, 1959), in vivo growth measurements having to await
the development of the more reliable agar media (Dubos and
Middlebrook, 1947; Middlebrook and Cohn, 1958) and efficient
organ homogenation methods (Fenner et al., 1949; Dubos
et al., 1953) These advances permitted an assessment of the
immune response in sublethally infected mice (Pierce et al.,
1953). By following the growth of the virulent challenge
organism in the lungs and spleens of intravenously or
aerogenically infected mouse strains, it is also possible to
detect substantial differences in host susceptibility in
relation to the H-2 antigenic background of the host (Youmans
et al., 1959; Gray, 1961; Lynch et al., 1965; Youmans and
Youmans, 1972; Closs and Haugen, 1974).

2.11 Vaccines for tuberculosis

2.11.1 BCG Vaccine

BCG, the bacillus of Calmette and Guerin is the only
prophylactic agent used against tuberculosis. BCG was
developed as a vaccine against tuberculosis in 1921 (Guerin,
1957). A strain of bovine variety of M.tuberculosis
isolated from an infected calf was passaged on bile salt
enriched media until it became avirulent for guinea pigs (Moore, 1983). This loss of virulence was shown to extend to cattle and to man. First administered orally to human infants by Weill-Halle in 1921 (Irvine, 1957), this method has slowly been replaced by the intradermal route. The vaccine is administered today either by intradermal injection or by scarification.

Within a few years of its development, cultures were sent to a number of countries, notably to Denmark. Subsequently, cultures of the Copenhagen strain have been distributed and the Glaxo freeze dried BCG vaccine used in the United Kingdom is derived from this strain (Osborn, 1976).

The efficacy of BCG has been the subject of considerable controversy. The results of a number of major vaccine trials show that protection against pulmonary tuberculosis varies from 80% to none at all. Table 1 gives the results of some of the BCG trials for tuberculosis. Many explanations have been advanced for the disparate results, including claims that poor results seen in some studies were artifactual. In an attempt to reach a firm conclusion on the value of the vaccine, a major and well controlled trial was organised in the Chingleputtu region of South India. Seven and half years later it was reported that the incidence of disease was no lower among the vaccinated than among the non-vaccinated controls (Report: Tuberculosis Prevention Trial,
<table>
<thead>
<tr>
<th>Study group/area</th>
<th>Date of inception</th>
<th>Reference</th>
<th>Protection against tuberculosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Indians</td>
<td>1936</td>
<td>Stein and Aronson (1953)</td>
<td>63-92</td>
</tr>
<tr>
<td>Southern United States of America</td>
<td>1950</td>
<td>Palmer et al. (1958)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Comstock et al. (1976)</td>
<td></td>
</tr>
<tr>
<td>MRC Trial, U.K.</td>
<td>1950</td>
<td>Medical Research Council (1963)</td>
<td>79</td>
</tr>
<tr>
<td>India (Madanapalle)</td>
<td>1950-55</td>
<td>Frimodt-Moller et al. (1964)</td>
<td>60</td>
</tr>
<tr>
<td>India (Madras)</td>
<td>1968</td>
<td>Tuberculosis prevention trial (1980)</td>
<td>0</td>
</tr>
</tbody>
</table>
1979). Several reasons have been advanced to explain the variations in the various trials. These include the differences in the potency of the vaccine, genetic and nutritional factors, and the effect of prior contact with mycobacteria in the environment. Rook and Stanford (1979) have shown that there are two types of cell-mediated immune responses to mycobacteria, both of which produce positive skin tests. These are termed the Listeria-type and Koch-type reactions; the former is protective, but the latter is less so and may even be antagonistic to protection. The findings of Stanford and his colleagues (1981) showed that some forms of immunologically effective contact with mycobacteria induce the former reaction while others induce the latter. The effect of BCG is to induce a Listeria-type reaction or to boost the reactivity previously induced by environmental contact. Thus BCG may induce or boost protective immunity in some communities and will appear effective, or it will boost a necrotic type of reaction in others and will not appear protective (Stanford et al., 1981; Stanford and Rook, 1983). Again it may be predicted that BCG will afford protection in the latter communities if given early enough in life to preempt the effect of environmental exposure. In studies involving guinea pigs, Palmer and Long (1969) found that such exposure afforded some protection against infection by *M. tuberculosis*. Vaccination with BCG increased this immunity but never to a level above that inducible by BCG alone. It was therefore concluded that BCG appeared to work well in populations not exposed to environmental mycobacteria but
elsewhere the observed effect of the vaccine is diminished by the previously acquired natural immunity. Hart (1967), Guld (1971) and ten Dam et al., (1976) have refuted the explanation that prior infection with atypical mycobacteria is solely responsible for the low efficacy of BCG in some trials. These authors in Puerto Rico and Georgia Alabama trials, show calculations which demonstrate that, even accepting the figures for protective efficacy of an atypical infection, it is mathematically impossible for this one variable to explain the low apparent protection afforded by BCG in these trials. There is also no conclusive evidence to suggest that major differences in the vaccines were responsible for the different results (Stanford and Rook, 1983). Although there are variations in the clinical presentation of tuberculosis in Britain and in South India and indeed there are differences in the virulence of tubercle bacilli in the two regions, there are no such differences between Britain and the United States. Thus, it is difficult to see how variation in the tubercle bacilli themselves can explain the observed results.

The decision as to whether a mass BCG campaign is desirable in a given region depends on financial and organisational considerations as well as epidemiological ones. Safety is one factor to be considered. Complications following vaccination include local abscess and regional suppurative lymphadenitis, particularly in infants, localised or generalised disseminated lesions and keloid scars (Lotte
et al., 1984). In recent years there have been reports on the development of osteitis in BCG vaccinated children. The frequency of this complication has ranged from 0.1/100,000 (Advisory Committee on Immunization Practices, 1979) vaccinees to 20/100,000 (Dahlstrom and Sjorgren, 1977). The organism isolated from the osteitic lesions was confirmed as BCG and it failed to cause progressive disease when inoculated into guinea pigs. Although the reason for the development of osteitis is unknown, factors that have been considered are: site of vaccination, age of the vaccinee and the method of vaccination preparation. Fatal disseminated BCG infections have been reported (Sicevic, 1972) but are extremely rare and are probably restricted to immunodeficient persons.

AIDS has become a global epidemic, with > 100,000 cases officially reported in 140 countries and the estimated 5-10 million asymptomatic carriers of the human immunodeficiency virus (HIV), the etiologic agent of AIDS. With an increase in HIV infection in some developing countries, there has been a resurgence of tuberculosis, and concern has been raised about the indications, efficacy, and safety of BCG vaccination. Anecdotal reports of local reactions and disseminated disease have been described in HIV infected children and adults. In many of the areas that are presently affected with HIV infection, the prevalence rates of endemic infection with M.tuberculosis are high (Vieira et al., 1983; Pitchenic et al., 1984). In developing countries,
tuberculosis is one of the most frequent opportunistic infections seen in patients with acquired immunodeficiency syndrome (AIDS) (Paper et al., 1985; Quinn et al., 1986; Mann et al., 1986; Colebunders et al., 1987; Michalany et al., 1987). Traditionally, live vaccines have been contraindicated in children with immunodeficiency diseases because of the potential for disseminated infection with the viral or bacterial vaccine strain (Redfield et al., 1987; Sension et al., 1987).

Thus the occurrence of tuberculosis among patients with AIDS (Centers for disease control, 1986), among the homeless (Centers for disease control, 1985), and among the elderly in nursing homes (Stead et al., 1985) have all contributed to a rising incidence of the disease. This highlights the need for an improved vaccine against tuberculosis which preferably should be effective in killed form, since vaccination with BCG is not always effective.

2.11.1.1 Means by which BCG Protects against Tuberculosis:
In the British Medical Research Council (MRC) trial (Tuberculosis Vaccines Clinical Trials Committee, 1959) BCG showed a protective efficacy of about 80% against pulmonary tuberculosis. Its efficacy against tuberculous meningitis was 100%. This observation is in keeping in view of the hypothesis that the protective effect of BCG in humans stems from its interference with the hematogenous spread of bacilli (Ladefoged et al., 1976). This hypothesis was directly
supported by a series of studies in an animal model in which guinea pigs were infected via the respiratory route with small numbers of tubercle bacilli. The bacillemic phase of the infection was delayed in onset and markedly reduced in extent in animals which received a potent BCG vaccine (Smith et al., 1979b).

A corollary of the hypothesis that BCG vaccination protects against human tuberculosis by interfering with the bacillemia accompanying the infection is that BCG vaccination would not be expected to influence the establishment of natural primary infection. This corollary is supported by autopsy findings which showed that virtually all tuberculous infections led to the development of pulmonary foci irrespective of whether the individuals were vaccinated or not (Sutherland and Lindgren, 1979).

2.11.1.2 Limitations of BCG Vaccine:

1) It does not protect all the genetic strains of mice, and if analogy is extended to humans, it may explain the variable effect of BCG observed in various trials.

2) There have been reports on the reversion of BCG vaccine organism from its attenuated state to full virulence (Petroff et al., 1927).

3) Fear of vaccine per se causing disease.

4) BCG is known to initiate systematic infection in leukemic patients and immunosuppressed individuals.

5) With an increase in HIV infection in some developing
countries, there has been a resurgence of tuberculosis, and concern has been raised about the indication, efficacy and safety of Bacille Calmette Guerin (BCG) vaccination.

In general, the present safety record of BCG vaccine is excellent and the fear of the vaccine causing progressive disease should no longer be a major factor in BCG controversy.

2.11.2 Vole bacillus vaccine

Results of studies by Wells and Brook (1940) and Birkhaug (1944) on guinea pigs, and of Griffith and Dalling (1940) and Young and Paterson (1949) on calves, indicated that a live vaccine made with the vole bacillus could give rise to immunity equal to or even greater than BCG. In Czechoslovakia and Britain, vole vaccine compared very favourably with BCG (Report, 1972). Its use was discontinued as some batches of vole vaccine caused a small number of cases of lupus 1-2 years after vaccination (Frew et al., 1955). These batches, although protective, induced a high degree of tuberculin hypersensitivity. Another batch was equally protective but induced only a low degree of tuberculin hypersensitivity and did not cause lupus (Report, 1971, 1972). The MRC Tuberculosis Vaccines Clinical Trials Committee concluded that it was worth considering the reintroduction of the vole vaccine (Report, 1972).

2.11.3 Other candidate vaccines
BCG is the only vaccine used in the prophylaxis of tuberculosis, but it has its own virtues and shortcomings as has been discussed. There is a long felt need to augment the immunogenic potential of BCG with other mycobacteria and/or search for more potent immunogenic strains.

Inactivated tubercle bacilli do not induce substantial levels of cell mediated immunity unless they are incorporated into water-in-oil type of emulsion with Freund's adjuvant and injected s.c. in two separate doses (Collins, 1973). At present, however, such adjuvanated preparations are too toxic for human use (Azurin et al., 1967). One advantage of a non-replicating vaccine would be that the antigens of the highly virulent \textit{M.tuberculosis} strain could be used in man. Such organisms may possess quantitative or even qualitative differences from the attenuated BCG, which could be expected to provide enhanced immune responsiveness to the vaccinated host. However, the virulence of such strains prevents their use in man as living preparations, while the lack of immunogenicity of saline suspension of the killed organism makes its use in this form unprofitable.

Earlier workers have attributed substantial antigenic activity to the phospholipids of tubercle bacilli. Crowle (1958) reviewed the modest protective effects of vaccination with methanol extract of \textit{M.tuberculosis} designated "antigene methanolique". Pigretti and colleagues (1956) found that an immunologically active mycobacterial extract could be
separated into at least five components, including cord factor and several mannophosphoinositides (PIM). None of the individual components conferred protection in mice against challenge with tubercle bacilli. The reconstituted mixture, however, regained almost complete protective capacity. However, Khuller and Subrahmanyam (1971) reported the stimulation of antibody production in rabbits inoculated with PIM in Freund's incomplete adjuvant. PIMs were also reported to be protective against tuberculosis in guinea pigs as assessed by body weight and mortality rate (Khuller et al., 1983). The sulpholipids of mycobacteria have also been reported to enhance resistance to M. tuberculosis H37Rv infection (Khuller et al., 1982). Lyophilised cell walls of M. bovis and M. tuberculosis H37Ra stimulated resistance in mice, challenged with viable M. tuberculosis H37Rv cells (Brehmer et al., 1968).

Larson and Witch (1964) have shown that live avirulent variant of M. tuberculosis, i.e. H37Ra is as effective as BCG in protecting mice against pulmonary infection with virulent tubercle bacilli when approximately 1x10^5 bacilli were employed. On the contrary, Collin's group (1969, 1973) in their protection studies with M. tuberculosis (H37Ra) indicate little or no antituberculous activity of M. tuberculosis H37Ra against a subsequent challenge.

Various other mycobacterial species have also been tested for their ability to immunize mice against subsequent
tuberculous challenge. Fenner (1957) first reported the increased survival of *M. tuberculosis* challenged mice which were preinfected with *M. avium*. Later Youmans *et al.* (1961) reported significant cross-protection of mice immunized with *M. avium*, *M. intracellulare*, or *M. kansasii* and later challenged with virulent tubercle bacilli. They detected no protection when scotochromogens and rapid growers were used to immunize the mice. More recently Siebenmann and Barbara (1964) and Pejovic *et al.* (1968, 1969) also observed significant antituberculous resistance in some *M. intracellulare* infected mice. This is in contrast to the findings of Larson and Witcht (1963) who had observed little cross-protection in mice vaccinated with this organism.

Collin, (1971) immunized CD.1 mice with $1 \times 10^6$ viable bacilli of *M. bovis* (BCG strain Tice, TMC 1028) or *M. kansasii* or *M. avium* or *M. intracellulare* or *M. terrae*, or *M. fortuitum* or *M. scrofulaceum*. The phylogenetic relationships between the vaccine and challenge strains had little direct influence on the level of resistance. A better correlation was found between the ability of the vaccine to survive or multiply in vivo and the immunogenicity of the organism. *M. bovis* (except for an $\text{SH}^R$ variant), *M. avium*, two of three *M. kansasii* strains, and one strain of *M. intracellulare* all survived or multiplied in vivo. They induced effective resistance against a subsequent *M. tuberculosis* (Erdman) challenge. The $\text{SH}^R$ BCG, the Bostrom strain of *M. kansasii* and *M. scrofulaceum* were unable to survive in vivo and were non allergenic and
nonimmunogenic.

Gupta et al., (1979) have tested the potential ability of *M. habana* and 18 other mycobacterial species to induce protection against 1 mg (1x10^7 bacilli) challenge dose of *M. tuberculosis* H37Rv in mice. The degree of protection conferred was assessed mainly by the survival time and necroscopy score. They showed the ability of *M. habana* to protect mice against tuberculous challenge when the live antigen was used by subcutaneous route. This immunogenic potency was further augmented when 0.1 ml of IFA was added to the inoculum. Out of the 19 mycobacterial strains used for vaccination, the only other strains found immunogenic were *M. tuberculosis* H37Ra, and *M. bovis* BCG, a finding similar to that of Youmans and Youmans (1965) and Collins (1971). The addition of IFA as such is not advisable for human usage.

Studies have been conducted by Youmans and Youmans in collaboration with Good (1977) which show that mycobacterial RNA preparations alone are highly effective immunizing agents in guinea pig. The results showed that the RNA vaccine exerted a good protective effect against challenge with the tubercle bacilli, although the protective effect did not quite equal that shown by the viable H37Ra cells. It is quite possible that the utilisation of larger doses of vaccine or the administration of multiple injections of vaccine might raise the level of immunity to that observed when guinea pigs are vaccinated with viable cells of BCG or the H37Ra strains.
However, extraction of mycobacterial ribosomal RNA and preparation of RNA vaccines from *M. tuberculosis* H37Ra or BCG is a cumbersome process as compared to the use of whole bacillus.

### 2.11.4 Recombinant DNA and subunit vaccines

Normally, the purification of antigens from *M. tuberculosis* would require their extraction from bacilli which is a major biochemical undertaking requiring large number of *M. tuberculosis* organisms. However, the advent of recombinant DNA technology has provided a major advance in identification, isolation and structural analysis of defined protein moieties having antigenicity. Genomic libraries of *M. leprae*, *M. tuberculosis* and *M. bovis* BCG were constructed by several workers (Clark-Curtis *et al.*, 1985; Thole *et al.*, 1985; Young *et al.*, 1985a, 1985b; Jacobs *et al.*, 1986; Khandekar *et al.*, 1986; Cohen *et al.*, 1987b). In a major step forward Young *et al.*, (1985a, 1985b) made *M. tuberculosis* and *M. leprae* libraries in a bacterial expression vector λgt11, which allows the expression of mycobacterial protein antigens in the bacterial host *E. coli*.

Screening of these genomic libraries with a large number of mouse monoclonal antibodies raised against *M. tuberculosis* (Engers *et al.*, 1985, 1986) resulted in the identification of genes for mycobacterial antigens which have immunodominant epitopes. Using this approach (Huynh *et al.*,}
genes for 71, 65, 19, 14, 12 KDa antigens of *M. tuberculosis* have been identified. The gene for 65 KDa antigen has been subcloned. Screening of this mini library with different anti-65 KDa monoclonal antibodies led to the identification of antigenic epitopes which are specific for the 65 KDa antigen of *M. tuberculosis* (Mehra et al., 1986; Shinnick, 1987). The 65 KDa antigen appears to be a major immunoreactive protein during the course of infection with either *M. leprae* or *M. tuberculosis*, as well as after vaccination with BCG. Both antibody and T cells directed against the antigen can be detected in the sera of patients and vaccinated persons, and response appears to be directed against specific as well as cross-reactive epitopes present on the 65 KDa antigen (Britton et al., 1985; Ivanyi et al., 1985; Thole et al., 1985; Emmrich et al., 1986; Lewis et al., 1986; Mustafa et al., 1986; Oftung et al., 1987). Furthermore, the genes for 65 KDa antigen of *M. tuberculosis*, *M. leprae* and *M. bovis* BCG have been sequenced and a comparison of the three sequences showed a high degree of homology. Thus, by making use of the recombinant methods, it is now possible to define an antigenic epitope reactive with either specific antibodies or T cell clones of interest. The recombinant proteins can be of diagnostic or prophylactic value.

The pathogenicity of mycobacterial infections and immunity to them are thought to be mediated by the host cellular immune response, which is directed against a number
of mycobacterial antigens. The 65 KDa has been found to be one of the major immunologically active mycobacterial antigens following infection or immunization. This antigen was originally identified in extracts of Mycobacterium leprae as the 65 kDa target of monoclonal antibody ML IIIE9. Young et al., (1988) have found that two of the M. tuberculosis protein antigens (19 kDa and 65 kDa) exhibit striking similarity to known stress proteins which are homologues of the E.coli dnaK and groEL proteins. While the function of stress proteins is not entirely clear, it appears that some participate in assembly and structural stabilisation of certain cellular and viral proteins (Neidhardt and VanBogelen, 1987; Pelham, 1986) and their presence at high concentrations have an additional stabilising effect during exposure to adverse conditions. Phagocytic host cells produce a hostile environment for foreign organisms, and the ability to produce stress proteins has been implicated in the survival of bacterial pathogens within macrophages. Young et al. (1988) have postulated that these abundant proteins are then among the major antigens available for presentation to T lymphocytes, and that this may contribute to their antigenicity.

Recent studies have shown stress proteins to be common immune targets in a broad spectrum of infectious diseases. Sequence analysis has revealed hsp70 homologues among major antigens of the protozoan parasites Plasmodium falciparum (Bianco et al., 1986) and Schistosoma mansoni (Hedstrom
et al., 1987) and the filarial parasite Brugia malayi (Selkirk et al., 1980). Similarly homologues of groEL have been found among antigens involved in the immune response to Salmonella typhimurium and Coxiella (Vodkins and Williams, 1988). The presence of stress proteins in a variety of human pathogens suggests that the stress response may be a general component of infection, and the stress proteins should be considered among candidates for subunit vaccines.

Ottenhoff's group (1988) has suggested that T cytotoxic cells (Tc) may have a pivotal role in the actual protection against mycobacterial infections. The recombinant 65 Kd hsp of BCG expresses immunodominant epitopes for peripheral Tc cells (Ottenhoff et al., 1988) as well as B cells (Young et al., 1987; Thole et al., 1988) and T helper cells (Emmrich et al., 1986; Young et al., 1987; Thole et al., 1988) in a significant number of individuals leading to the suggestion that this protein seemingly may have potentials of a subunit vaccine. Moreover, because the stress response is common to prokaryotes and eukaryotes and some stress proteins are highly conserved in sequence (Bardwell and Craig, 1984, 1987; Lindquest, 1986), it is possible that an antigen from one pathogen could immunize against another pathogen. Immunization with conserved protein antigens, whether during natural infection or during vaccination, might also have negative consequences. Presence of a self-like determinant may fail to stimulate lymphocytes due to host tolerance. Chronic presentation of such determinants might occasionally result in breaking of tolerance and production of an
autoimmune response.

The vaccines used till today for immunization against tuberculosis in humans or experimental animal models have been either live nontuberculous or atypical mycobacteria. There have been reports by various workers on the diseases associated with *M. fortuitum*, *M. kansasii*, *M. avium intracellulare*, *M. scrofulaceum* etc. in humans (Wolinsky, 1984). BCG, the conventional vaccine, does not immunize all the genetic strains of mice and if analogy is extended to humans it might explain the variable effect of BCG observed in different populations. Hence, a need is posed for finding a non-viable vaccine which is able to protect both the responder and non-responder genetic strains of mice and humans of different immunogenetic backgrounds.

2.11.5 *Mycobacterium w* as an anti-leprosy vaccine

*Mycobacterium w* is an atypical, non-pathogenic and rapid growing bacteria. It is niacin negative and positive for semi-quantitative catalase and heat resistant catalase. It lacks the ability to hydrolyse Tween-80 up to ten days but degrades sodium salicylate. It reduces nitrite to nitrate. It grows on Lowenstein-Jensen, Middlebrook and Sauton’s medium. The organism does not grow on nutrient agar or McConkey’s Agar. It does not form pigment either in the dark or in the light (Saxena *et al.*, 1978; Katoch, 1981). Many of these properties have also been counter checked by Dr. Kristina Wickman at the National Bacteriological Laboratory,
Figure 1: Scanning Electron Micrograph of *Mycobacterium w.* (Magnification: 10000x).
Stockholm, Sweden. On the basis of its growth and metabolic characteristics, it is classified in Runyon's group IV. However, it differs from other strains previously included in this group.

Fifteen strains of cultivable mycobacteria were screened in terms of their potential antigenic similarity to M. leprae. Amongst these, M. w and a slow growing ICRC strain related to M. avium intracellulare group were found to fulfill these criteria.

Studies on M. leprae revealed that the peripheral blood mononuclear cells (PBMC) from tuberculoid leprosy patients proliferated in response to this organism in a manner analogous to M. leprae (Mustafa and Talwar, 1978a). Immunization of guinea pigs with M. w and M. leprae induced homologous and cross-reactive DTH skin response (Mustafa and Talwar, 1978b). Like killed M. leprae, injection of mice with M. w caused chronic enlargement of draining lymph nodes (Mustafa and Talwar, 1978c), and long lasting delayed infiltration of mononuclear cells in the mouse foot pad (Fotedar et al., 1978). Lepromin-like preparations prepared from M. leprae and M. w gave comparable DTH skin responses in tuberculoid leprosy patients (Girdhar and Desikan, 1978; Govil and Bhutani, 1978; Hogerzeil and Prabhudass, 1978; Sahib and Vellut, 1978; Sharma and Singh, 1978).

Intradermal immunization with M. w converted a large percentage of lepromin negative lepromatous leprosy patients
to lepromin positive status (Chaudhuri et al., 1983). These studies showed M.w to be a potent immunogen having antigens of considerable cross-reactivity with M.leprae antigens in CMI functions.

In recent studies Mustafa (1988) demonstrated that M.w has antigens that cross-react with at least three major protein antigens of M.leprae. However, M.w has some antigens unique to itself. The M.leprae cross-reactive as well as non cross-reactive antigens of M.w may play a role in the induction of protective immunity to M.leprae infection in the subjects vaccinated with M.w.

Mustafa et al., (1988) have also demonstrated that M.w has antigens which cross-react with 65 KDa and 19 KDa proteins of M.tuberculosis. Studies carried out in our laboratory indicate that M.w cross-reacts with M.tuberculosis at B cell level also. This was shown by immunoblot studies with monoclonal antibodies specific to or cross-reactive to M.tuberculosis and with tuberculosis patient sera (Ganju et al., 1990).

*Mycobacterium* w was selected as a candidate leprosy vaccine based on the rationale previously described. After extensive toxicology trials and approvals by the Drug Regulatory Authorities, a vaccine based on M.w is currently in Phase II/III immunotherapeutic trials in leprosy patients. The vaccine has been shown to expedite bacterial clearance and bring about clinical improvement in a manner superior to
drugs alone. Multibacillary patients become bacterial negative at a distinctly accelerated pace. Furthermore, they become lepromin positive. Upgrading of the immunological status is also indicated at histopathological level (Talwar et al., 1989; 1990a; 1990b). The vaccine is now in field use in a population of 360,000 people in Kanpur Dehat, U.P., India.

The present study investigates the ability of M.w to confer protection against tuberculosis in experimental animal models, mainly mice (responder and non-responder strains to BCG) and guinea pigs.