REVIEW OF LITERATURE
2.1 Historical Overview

Leprosy is an ancient disease and has been mentioned in the Vedas as long ago as 1400 B.C. Sushurata in 600 B.C. described it as a contagious disease. The disease was probably carried from India to Europe in the fourth century B.C. by returning soldiers and camp followers from the Greek wars of conquest in Asia led by Alexander the Great. The disease slowly spread throughout Europe carried by soldiers, traders and settlers. It was most active in Western and Northern Europe between the 10th and 15th centuries. Moller-Christensen (1961) demonstrated classical leprosy in skulls and bones excavated from the burial ground of a lazaret hospital which existed between 1250 and 1550 A.D.

*Mycobacterium leprae* the causative organism was first described by Hansen in 1873 (his observations were published in 1874) in fresh material obtained from a human lesion and treated with osmic acid. Neisser (1879) subsequently succeeded in staining the organism with fuchsin and gentian violet. The organism is a pleomorphic acid fast rod, with mean length of 2.1 um and mean width of 0.25 to 0.30 um (Draper and Misell, 1977). In stained tissue smears they occur either singly or in bunches or in compact masses known as "globi". *M. leprae* is less acid fast than *M. tuberculosis*, therefore, a weaker acid-alcohol mixture is used for a shorter time to decolourize the organism. *M. leprae* is the only myco-
bacterial species which loses its red colour when the smears are treated with pyridine.

2.2 Epidemiology

Leprosy has a world-wide distribution and is most prevalent in the tropics and subtropics but it also occurs as an endemic disease in some temperate regions. The total number of leprosy cases in the world was estimated to be between 10 and 11 million by Sansarricq in 1983. India with an estimated 4 million cases (1981) accounts for about one-third of the total leprosy cases of the world and half the cases in Asia. Of the 4 million cases 20-25% are of the multibacillary type. The country has an overall prevalence rate of about 5 cases per 1000 but in some areas it is as high as 40 per 1000 (Park and Park, 1986).

Although there are reports on the presence of M.leprae in wild armadillo (Smith et al., 1983) and spontaneous appearance and transmission of M.leprae in chimpanzee (Donham and Leninger, 1977) and a mangaby monkey (Meyers et al., 1980) leprosy is still considered a strictly human disease with man as the main reservoir of M.leprae and therefore the major source of infection. The disease has an incubation period varying from 2-5 years which could be due to a very slow generation time of almost 2 weeks in contrast to 20 hours of M.tuberculosis (Shepard, 1971). Due to this long incubation period, the exact mode of transmission of this
disease remains largely unknown. However, the most probable route is mucosa of the upper respiratory tract. The chief portal of exit of \textit{M. leprae} from the human host is thought to be the nasal mucosa of the LL patients. About 72\% of the LL patients harbour \textit{M. leprae} in the nasal mucosa (Pedley, 1973). Approximately $10^7$ to $10^8$ bacilli are discharged daily from the nasal mucosa as well as the skin ulcers of the lepromatous patients. A sneeze at close distance can transmit enough bacilli for infection of a mouse (Davey and Rees, 1974). The bacilli from nasal discharge remain viable for several weeks in moist soil (Desikan and Sreevastav, 1979). \textsc{tt/bt} patients ordinarily do not excrete \textit{M. leprae}. However, in rare instances, during an acute exacerbation of the disease, the lesions ulcerate and nerve abscesses are formed, discharging \textit{M. leprae} along with pus (Dharmendra and Guinto, 1985).

Skin transmission by arthropods (flies and mosquitoes) has also been reported. In fact, Narayanan \textit{et al.} (1977) reported the transference of \textit{M. leprae} from humans to mouse foot-pads by mosquitoes.

2.3 Pathogenesis

Only 10-20\% of those exposed to \textit{M. leprae} get indeterminate leprosy. Of these about half progress to full blown clinical leprosy (Miller, 1987). The involvement of the peripheral nervous system (PNS) is an essential feature of the disease (Job and Desikan, 1968). The regions of
the nerve which are at slightly lower temperature and where they are more liable to trauma, are the most vulnerable. The extent of damage to the nerves and the spread of infection depends on the immune status of the host. In the host with good immune response e.g. patients with tuberculoid leprosy (TT), the nerve is invaded by lymphocytes and histiocytes (macrophages) and finally a tuberculoid granuloma is formed. The granuloma leads to thickening and destruction of the nerves and results in anaesthesia and muscle weakness. The infection may be confined to the nerves but following the escape of the bacilli into the skin, a skin lesion may develop. On the other hand, in the host with depressed cell-mediated immune (CMI) response e.g. lepromatous leprosy (LL), the bacilli multiply unchecked in the Schwann cells and also cause perineurial damage (Pearson and Ross, 1975). Large numbers of bacilli are liberated by the destruction of these cells. These bacilli are engulfed by macrophages and inside these wandering cells the bacilli multiply. The macrophages carry the bacilli to other parts of the nerve and to other tissues via blood, lymph and tissue fluid. These are the individuals who are likely to get bacillemia. Destructive lesions are seen in the skin, peripheral nerves, eyes, upper respiratory tract, testes and structures of hand and feet. The predilection of M. leprae to these tissues may be due to the fact that they are relatively cooler than
the deep lying tissues.

2.4 Clinical spectrum of leprosy

Clinically leprosy is not a single entity but manifests itself as a spectral disease (Ridley and Jopling, 1966; Sansonetti and Lagrange, 1981; Bloom and Godal, 1983). This variation is not due to bacterial strains of varying pathogenicity, as *M. leprae* from patients with different types of leprosy show identical behaviour when injected into susceptible strains of mice (Rees, 1969). It can, however, be correlated to the immune status of the individual (Ridley and Jopling, 1966). Ridley and Jopling (1966) gave a formal classification of the disease. They classified the patients into five categories taking into account the immunological and histopathological aspects, in addition to the clinical and bacteriological findings. These categories are tuberculous (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL) and lepromatous (LL). The BB, BL and LL patients are also referred to as multibacillary patients whereas the BT and TT are grouped together as paucibacillary leprosy.

The LL patients who form one end of the spectrum have several lesions and have a high load of bacilli. These patients show a poor CMI response to the organism but have a high level of anti-*M. leprae* antibodies. The TT patients occupy the other end of the spectrum. They are defined clinically by the presence of 1-3 well defined
lesions. Very few, if any, bacilli can be detected. These patients have a strong CMI response to *M. leprae* antigens and a low level of anti-*M. leprae* antibodies. The borderline (BL, BB and BT) patients fall in between these two polar groups. They can either upgrade to the tuberculoid end following an upgrading reaction or go to the lepromatous end after a downgrading reaction. Indeterminate leprosy is an early and transitory stage of leprosy found in persons whose immunological status has yet to be determined. This form of disease may remain quiescent, heal spontaneously or may progress insidiously into one of the determinate types in the spectrum. These patients can also be labelled as "pauci-bacillary leprosy" patients.

During the course of the disease the borderline patients may undergo a "reversal" or "upgrading" reaction due to an increase in CMI or they may downgrade (downgrading reaction) due to a decrease in CMI. These are known as the Type I reactions. The LL and occasionally BL patients, on the other hand, can undergo the Type II or Lepra reaction due to the formation of immune complexes (Moran et al., 1972).

### 2.5 Diagnosis of leprosy

#### 2.5.1 Conventional diagnosis

The diagnosis of leprosy in a suspected case is done by a detailed clinical and histopathological examination. The cardinal signs of leprosy are anaesthesia, thickened
nerves, skin lesions and AFB in (slit) skin smears. The number of AFB are more in lepromatous smears and virtually absent towards the tuberculoid end.

2.5.2 Immunodiagnosis

Several groups have used assays based on the detection of antibodies in sera of leprosy patients. Abe et al. (1976) used intact *M. leprae* for the detection of antibodies in leprosy patients by immunofluorescence. Harboe et al. (1978) reported a radioimmunoassay using sonicate filtrate of *M. leprae*. Cho et al. (1983) reported the use of phenolic glycolipid-I (PGL-1) for the detection of leprosy. A gelatin particle agglutination test (MLPA) using the terminal synthetic trisaccharide of PGL-I was developed for the serodiagnosis of leprosy by Izumi et al. (1990). Other groups have used cross-reactive mycobacteria such as *M. smegmatis* (Dougals et al., 1984; Miller et al., 1983), *M. fortuitum* (Vithasyasai et al., 1983) *M. w* (Moudgil et al., 1988; Ganju et al., 1988) for the detection of antibodies. Monoclonal antibodies specific to *M. leprae* antigens have been used in competitive assays (Sinha et al., 1983; Klaster et al., 1985). Species-specific serological responses have been demonstrated to a range of peptide epitopes specific to *M. leprae*. These include the 35KDa (Sinha et al., 1985), 36KDa (Klaster et al., 1985) and the 18KDa (Britton et al., 1985) protein antigens of *M. leprae*. Das et al. (1990) have used *M. tuberculosis* 29/33K and 64K antigens.
to distinguish between lepromatous and tuberculoid forms of the disease.

The major limitation with most of the antibody-based assays was their inability to pick up the paucibacillary cases. All the assays reported had a good sensitivity for multibacillary cases but except for the fluorescent leprosy antibody absorption (FLA-ABS) test (Abe et al., 1980, 1981; Bhardwaj and Katoch, 1989; Baohong et al., 1984; Amezcua et al., 1987) and more recently an enzyme immunoassay reported by Das et al. (1990), their utility for the diagnosis of paucibacillary cases was fairly low.

Assays based on the detection of antigen have been reported in serum, urine, and tissues. Detection of PGL-1, which is an M. leprae specific antigen has been reported by Young and Buchannan, (1983) and Cho et al. (1986) in serum and urine of LL/BL patients and by Kaldany and Nurlign (1986) in urine. Olcen et al. (1983) have used an inhibition assay for the detection of M. leprae antigens in the urine of LL patients.

2.5.3 Nucleic acid probes

Clark-Curtiss and Docherty (1989) described a DNA probe that can be used in dot-blot hybridization assay to detect as little as 1 pg of purified DNA—the amount present in 300 bacilli. Woods and Cole (1989) have described a PCR-based assay in which a single pair of primers to a portion of M. leprae groEL gene or a single
A pair of primers to a portion of *M. leprae* repetitive sequences has been used. Similarly, Hartskeel *et al.* (1989) used a single pair of primers to amplify a portion of gene encoding the *M. leprae* 36KDa antigen from infected armadillo tissue. Plikaytis *et al.* (1990) developed a two-step PCR assay to detect *M. leprae* based on amplification of a portion of *M. leprae* groEL gene using a set of four-nested oligonucleotide primers. This assay could pick up 3 fg of *M. leprae* genomic DNA which is the amount present in a single bacillus.

2.6 *M. leprae* antigens

Due to its complex nature, it has not been possible to define the exact number of antigenic determinants in *M. leprae*. In 1980 Closs *et al.* demonstrated up to 90 antigens using the technique of crossed immunoelectrophoresis. This, however, may not be the total number of antigens present in *M. leprae*.

More recently a number of biochemical, immunological and molecular biological techniques have been used to identify and characterize several cytoplasmic and cell wall associated protein and lipid antigens of *M. leprae*.

2.6.1 Glycolipids/Glycoconjugates

Phenolic glycolipid-I (PGL-I) and lipoarabinomannan B (LAM-B) are the two most extensively studied glycolipid antigens of *M. leprae*. Both these antigens, which are associated with the cell wall of the organism, have been found to influence the immune response in leprosy. PGL-I
was first reported by Hunter and Brennan (1981) and its surface localisation was demonstrated by Young et al. in 1984 using monoclonal antibodies. Mehra et al. (1984) reported that PGL-1 is a potent inducer of suppressor lymphocytes in lepromatous leprosy patients. It has also been shown to scavenge reactive oxygen intermediates and contribute to the survival of the leprosy bacillus (Holzer et al., 1986; Neil and Klebanoff, 1988).

LAM-B, the other highly immunogenic glycolipid of M.leprae is a prominent component of the cell wall and is also present in M.tuberculosis and other mycobacteria. It has been shown to be a potent inhibitor of IFN-γ mediated activation of mouse macrophages in vitro (Sibely et al., 1988). It has also been shown to bring about suppression of lymphocyte proliferation in vitro. Since this effect has been seen both in healthy exposed/unexposed individuals and all categories of leprosy patients, it has been inferred that the suppressive effect is due to the leprosy bacillus per se (Kaplan et al., 1987). Sera from leprosy patients including those with tuberculoid leprosy contain high titre antibodies to LAM (Chakrabarty et al., 1982; Miller et al., 1983; Brennan, 1986). Gormus et al. (1990) suggest that anti-LAM antibody levels appear to be potentially valuable as an indicator of leprosy susceptibility.
2.6.2 Protein antigens

2.6.2.1 Heat shock proteins

Several cross-reactive and species-specific epitopes have been identified on *M. leprae* using monoclonal antibodies (Engers, *et al.*, 1985, 1986; Gaylord *et al.*, 1987). Most of the laboratories attempting to produce monoclonal antibodies against *M. leprae* have identified six *M. leprae* proteins with Mr of 70, 65, 36, 28, 18 and 12 KDa. Almost all these proteins have been expressed from DNA libraries and most of them have been sequenced (Mehra *et al.*, 1986). Three of these immunodominant protein antigens viz. 70KDa, 65KDa and 18KDa have now been identified as stress or heat shock proteins (hsp's). Out of these, the 65 KDa protein has been extensively studied and has been found to be associated with the isolated cell wall of the bacillus (Gillis *et al.*, 1985). It belongs to the family of highly conserved bacterial proteins represented by the 65KDa product of the *groEL* gene in *E. coli* (Young *et al.*, 1988 a&b). It has 95% amino acid homology with the corresponding gene product of *M. tuberculosis* and *M. bovis* (Shinnick *et al.*, 1987, 1988) and approximately 50% homology with the *E. coli* groEL and with the corresponding protein from human cells. Studies by different groups have shown the presence of several T and B cell epitopes on this protein (Emmrich *et al.*, 1986; Lamb *et al.*, 1987; Thole *et al.*, 1988; van Schooten *et al.*, 1988). Anderson *et
al. (1988) reported an M. leprae-specific T cell clone derived from a tuberculoid patient recognising an epitope on the 65KDa antigen, which cross-reacted with a peptide derived from the third hypervariable region of HLA-DR2 chain. They suggest that this cross-reactivity may have a role in the pathogenesis and immunopathology observed in tuberculoid leprosy. The 180-188 residues of the mycobacterial 65KDa antigen have also been identified as the triggering agent of adjuvant-induced arthritis in rats (van Eden et al., 1988). It has been suggested that the recognition of antigenic determinants conserved between mycobacterial and human proteins may play a role in immunopathology seen in tuberculoid patients (Young et al., 1988).

The 70 KDa antigen of M. leprae is homologous with the E.coli dnaK gene product, having about 55% homology with the carboxy terminus of this protein, and about 92.5% homology with the carboxy terminus of the 71KDa protein of M. tuberculosis (Britton et al., 1986). It shows strong reactivity with sera from leprosy and tuberculosis patients (Britton et al., 1987, 1988). Affinity purified BCG 70 KDa protein induced T cell responses in leprosy patients, their contacts and in Montoux positive subjects (Britton et al., 1986). M. bovis 70 KDa protein secreted in large amounts into the culture filtrates has been found to bind ATP and it has been suggested that like other proteins of the 70 KDa family from other...
organisms this protein might possess a functional ATPase activity (Garsia et al., 1989).

The 18KDa protein sequence has been found to be similar to the soyabean 17KDa hsp (Nerland et al., 1988). Mustafa et al. (1986) first reported the presence of T cell epitopes on this antigen. Subsequently several other workers have reported the presence of both T and B cell epitopes on this protein (Booth et al., 1988; Dockrell, et al., 1989; Harris et al., 1989; Doherty et al., 1989). The 18 KDa antigen of M.leprae was thought to be an M.leprae-specific antigen, however, recent studies on M.w and M.habana show the presence of this molecule in these mycobacteria also (Mustafa, 1988 1990; Ganju et al., 1990; Lamb et al., 1990).

2.6.2.2 Other protein antigens

Young et al. (1985) reported the presence of a 28 KDa protein antigen on M.leprae using monoclonal antibodies. They could identify species-specific and cross-reactive epitopes on this antigen. Cherayil and Young (1988) cloned the gene for a 28 KDa antigen from Agt11 M.leprae DNA expression library. This protein was picked up by screening the library with pooled sera from lepromatous patients. The sequence of this 28 KDa protein is different from the 28KDa reported by Young et al. (1985). It's sequence suggests that it is targeted for export from the cytoplasmic compartment of the cell. They suggest that a part of this protein could be associated with the
cell wall and this could have implications for the role of this protein in the immune response in leprosy as most of the humoral and cellular immunogenicity of mycobacteria is associated with the cell wall (Melancon-Kaplan et al. 1988). Thangraj et al (1990) have sequenced a 28 KDa protein from *M. leprae* which has sequence homology with the highly conserved superoxide dismutase.

The 36 KDa antigen of *M. leprae* was originally identified by virtue of its reactivity with leprosy patients' sera by Klaster *et al.* in 1984. Later the gene coding for this protein was picked up from the *gtl1* library with the help of monoclonal antibodies (Klaster *et al.* 1990). The DNA sequence coded for a polypeptide of 249 amino acids with a predicted molecular mass of 26,299 daltons. The deduced amino acid sequence revealed a proline rich amino terminal region. It has been reported to stimulate both helper (Ottenhoff *et al.*, 1986; van Schooten *et al.*, 1988) and suppressor (Ottenhoff *et al.*, 1986) T cell clones. de Wit and Klaster (1988) again reported that sera from a majority of leprosy patients contained antibodies recognising this antigen.

Melancon-Kaplan *et al.* (1988) reported the immunological significance of mycobacterial cell walls. Purified cell walls were capable of stimulating proliferation of T cells from TT but not from LL patients. These were also
capable of eliciting DTH reaction in guinea-pigs and patients sensitized to \textit{M.\textit{leprae}}. Several immunodominant antigens were identified in these preparations using cell wall-specific \textit{T} cell clones from leprosy patients (Mehra \textit{et al}., 1989).

2.7 Immune response in leprosy

Infection with \textit{M.\textit{leprae}} is manifested as a clinical and immunological spectrum ranging from the localized, highly resistant \textit{TT} to multibacillary low resistant lepromatous leprosy (Ridley \& Jopling, 1966). Both humoral and cellular immunity appear to be operative after natural infection and experimental immunization with \textit{M.\textit{leprae}}. However, as \textit{M.\textit{leprae}} is an obligate intracellular parasite, the protective immune response in leprosy as in other mycobacterial diseases is of the cell mediated type. The humoral responses do not appear to play a role in protection.

2.7.1 Humoral immune response

The humoral immune response in general seems to be unimpaired in leprosy. The levels of antibodies to \textit{M.\textit{leprae}} vary along the spectrum. Patients with the polar or borderline tuberculoid form of the disease may have either low or no antibodies, whereas patients with borderline lepromatous or lepromatous leprosy invariably have high titers of antibodies to \textit{M.\textit{leprae}} (Myrvang \textit{et al}., 1974).
In an attempt to identify the immunodominant antigens of \textit{M.leprae}, Ehrenberg and Gebre (1987) using LL sera identified the 13.5 and 28 KDa antigens as the immunodominant antigens. Chakravarty \textit{et al.} (1982) identified a broad 33 KDa glycoprotein band with LL sera. Klaster (1984) in an attempt to identify \textit{M.leprae} specific proteins absorbed the sera of five lepromatous patients with other mycobacterial extracts and identified the 12, 22 and 33 KDa bands. Vega-Lopez \textit{et al.} (1988) immuno-blotted sera from leprosy patients and healthy individuals with \textit{M.leprae} sonic extract. They identified five proteins with relative molecular weights of 33, 25, 18, 15 and 12 KDa which were frequently recognized by the patients. de Wit and Klaster (1988) reported the recognition of the 36KDa antigen by the majority of lepromatous sera studied by them. Britton \textit{et al.} (1988) immunoprecipitated \textit{M.leprae} antigens with sera from untreated patients and found that the major \textit{M.leprae} antigens which were identified by this procedure were of low molecular mass (48, 36, 33, 27, 15 & 12 KDa). High levels of circulating antibodies have been reported to PGL-I by several groups (Cho \textit{et al.}, 1983, 1986; Young and Buchannan, 1983; Bach \textit{et al.}; 1986; Hussain \textit{et al.}, 1990; Meeker \textit{et al.}, 1990). Several other groups have reported the binding of patient sera to different molecular weight components of \textit{M.leprae}, BCG etc. Pessolani \textit{et al.} (1989) reported the recognition of a 28-30 KDa
doublet from early culture filtrates of *M. bovis* BCG by 92% of the LL sera. Similar results were obtained with sonic extracts of seven other slow and fast growing mycobacterial species. Das *et al.* (1990) could differentiate the LL and TT sera by their differential recognition of 29/33 and 64 KDa antigens respectively. Sera from leprosy patients have a dominant immune response to the *M. tuberculosis* 30/31K antigen which is a secretory protein and has been reported to bind fibronectin (Abou-Zeid *et al.*, 1988). Recombinant DNA approach has indicated that *M. leprae* probably expresses a homologous protein.

Cherayil and Young (1988) also reported the immunodominance of the 28 KDa protein of *M. leprae* recognised by LL sera. The 18 KDa antigen of *M. leprae* has also been reported to be a target for serological response (Doherity *et al.*, 1989).

2.7.2 **Cell mediated immune response**

After exposure to *M. leprae*, most people develop protective immunity to the bacillus and usually demonstrate a positive skin test (DTH) response with lepromin. However, as stated earlier, leprosy patients show evidence of depressed CMI, the degree of depression varying from the tuberculoid to the lepromatous pole with maximum depression being manifest in untreated lepromatous patients. Thus a strong DTH response is observed in tuberculoid patients, whereas, virtually no response is seen in the
lepromatous group. Similar results are seen in *in vitro* experiments analysing the cell mediated immune responses of the leprosy patients. There are reports demonstrating defects in both non-specific and specific CMI response. Decreased response to mitogens has been reported by several workers (Dierks & Shepard, 1968; Sheagren *et al.*, 1969; Bullock & Fasal, 1971). Some workers have reported a significant alteration in helper and suppressor T cell ratio with an increase of the T8+ cells and a decrease in the T4+ cells in the lepromatous patients (Mshana *et al.*, 1982; Wallach *et al.*, 1982) while others (Bullock *et al.*, 1982; Rea *et al.*, 1983) did not find any alteration.

2.7.2.1 Mechanism of T cell unresponsiveness in leprosy

The underlying mechanisms of defective CMI are still not clearly understood. A number of hypotheses have been put forward by several groups. These include defect at the level of killing of *M. leprae*, defective antigen presentation, absence of antigen-specific helper T lymphocytes, active suppression due to generation of specific suppressor T lymphocytes/factors, failure to produce T cell growth factors *etc*.

2.7.2.1.1 Killing of *M. leprae*

*M. leprae* resides in the macrophages of the mononuclear phagocyte system and the Schwann cells of the peripheral nervous system. The earliest reports of the involvement
of macrophages were by Barberi and Correa (1967) followed by Beiguelman (1971). They observed that blood-derived macrophages from LL patients were unable to cause the lysis of *M. leprae* in vitro while macrophages from the tuberculoid patients lysed the bacilli. Rojas-Espinosa (1978) has shown that lepromatous macrophages have a normal capacity to ingest *M. leprae*. In fact, Bullock *et al.* (1978) suggested that the lepromatous macrophages are refractory to normal regulatory mechanisms, resulting in excess phagocytosis. It was, therefore, speculated that this excessive phagocytosis would result in the neutralization of bactericidal mechanisms mediated by reactive oxygen intermediates and lysosomal enzymes, leaving a proportion of the bacilli free to exert their influence.

Although the mechanism of antigen processing by Schwann cells has not been studied, the mechanisms involved in the killing of any intracellular pathogen in the macrophage is well known. Following phagocytosis, the pathogen may prevent its own destruction by preventing phagosome-lysosome fusion or by interfering with the activity of lysosomal enzymes/generation of toxic reactive intermediates.

### 2.7.2.1.1.1 Phagolysosome

Inhibition of phagolysosome fusion in *M. leprae*-infected macrophages in vitro has been reported by Frehel and Rastogi (1987) and Sibley *et al.* (1987). The former
implicate the involvement of \textit{M. leprae} surface components in this process since treatment of \textit{M. leprae} with anti-\textit{M. leprae} antibodies before phagocytosis reversed this inhibition. Decreased lysosomal enzyme levels in LL patients as compared to TT and normal individuals have been reported by Marolia and Mahadevan (1984). However, in an earlier report, Avila and Convit (1970) and Miyata and Saito (1963) found normal enzyme levels in the peripheral blood-derived macrophages of LL and TT patients.

2.7.2.1.1.2 Oxidative burst

Hydrogen peroxide (H$_2$O$_2$) and Superoxide (O$_2^-$) have been reported to be mycobactericidal (Klebanoff and Shepard 1984; Sharp \textit{et al.}, 1985). However, since \textit{M. leprae} contains superoxide dismutase it is likely that it is able to protect itself from the effects of superoxide radicals (Wheeler and Gregory 1980; Colston \textit{et al.}, 1990). Further, the phenolic glycolipid I (PGL-I) of \textit{M. leprae} has been shown to scavenge reactive oxygen intermediates and this may prevent the bactericidal action of these oxygen radicals (Holzer \textit{et al.}, 1987; Neil and Klebanoff 1988). Marolia and Mahadevan (1987, 1988 & 1989) reported production of decreased amounts of superoxide in response to \textit{M. leprae} infection in macrophages from leprosy patients but not in normal individuals. This deficiency could be overcome in cells
from TT patients in the presence of lymphokines.

2.7.2.1.2 Antigen presentation

A defect at the level of presentation of antigen to T cells has also been implicated in the defective CMI. Hirschberg (1978) showed that T cells from non-responding patients in combination with macrophages from responding patients or human contacts did respond to *M. leprae*. Conversely, T cells from responding patients or healthy contacts in combination with macrophages from unresponsive patients failed to respond. It was therefore concluded that the lack of response observed in *in vitro* tests was seemingly due to a failure of their macrophages to present *M. leprae* antigens in an immunogenic form. This was corroborated by Nath *et al.* in 1980 using HLA-D matched siblings.

Watson (1975) noted a depressed immune response to sheep erythrocytes at an early stage of *M. lepraemurium* infection and attributed the cause to macrophages. His explanation of this immunosuppression was that overloading macrophages with mycobacteria could interfere with their ability to ingest, process and present other antigens.

Birdi *et al.* (1989) reported downregulation of Fc and Con A receptors and HLA-DR in macrophages from LL patients after infection with *M. leprae*. These macrophages were unable to respond to activation. These effects were
not observed with non-viable *M. leprae* suggesting that
*M. leprae* may be secreting certain products which bring
about these alterations (Wadee et al., 1980; Mehra et al., 1984).

Route of immunization has been implicated in the patho­
genesis of the non-responder state (Shepard et al.,
1982). Shroff et al. (1990 a&b) also observed a route
related variation in immunogenicity. Intraperitoneal
immunization with rapid growers led to a poor immune
response. They attributed it to defective antigen pre­
sentation as this non-responsiveness could be overcome
by prior administration of poly I:C, an IFN-γ enhancer
or indomethacin, a prostaglandin inhibitor.

2.7.2.1.3 **Role of helper T cells and cytokines in
leprosy**

One of the earliest hypotheses which was put forward to
explain the unresponsiveness in lepromatous leprosy
patients was absence of *M. leprae*-reactive T cells.
However, studies carried out by Haregewoin et al.
(1983), Nath et al (1984b), Kaplan et al. (1985) and
Shankar et al. (1986) demonstrated the presence of
*M. leprae*-reactive T cells in lepromatous patients. They
attributed the non-responsiveness of these T cells to
antigens of *M. leprae* to a defect in the production of T
cell derived factors such as IL-2 and IFN-γ. Earlier
studies by Bullock (1978) and Rook et al. (1976) on
M. lepraemurium infections suggested that there was a long term sequestration of antigen-reactive T cells in the lymph node. Desai et al. (1988) also reported the presence of M. leprae-reactive T cells in the lymph nodes of lepromatous patients. Hargewoin et al. (1983) first demonstrated that T cell unresponsiveness in LL patients could be restored \textit{in vitro} if the cells were cultured in the presence of T cell-conditioned medium. Nogueira et al. (1983) showed that IFN-\(\gamma\) release by lepromatous patients could be partially restored by culturing the cells with purified IL-2 and M. leprae antigens. Studies carried out by Nath et al. (1984 a) demonstrated that a proportion of LL patients possessed M. leprae-reactive T cells which could be induced to proliferate in the presence of exogenous human IL-2. Kaplan et al. (1985) also reported that a considerable proportion of LL patients exhibited a low degree of responsiveness to M. leprae. These responses could be enhanced by the addition of exogenous IL-2 or depletion of monocytes. However, non-responsiveness seen in a small proportion of LL patients could not be reversed by the addition of IL-2. Administration of human recombinant IL-2 intradermally into the skin of lepromatous patients with high bacillary load led to the generation of an effective cell mediated immune response. This in turn brought about striking local reduction in the bacillary load (Kaplan et al., 1989a).
Shankar et al. (1986) generated *M. leprae* and PPD-reactive T cell lines from TT and LL patients. They also observed that the addition of IL-2 increased the proportion of LL patients responding to both PPD and *M. leprae*. Trao et al. (1988) in a study on new and old cases of leprosy found that in contrast to the new patients, responses of only some of which were reconstituted by the addition of lymphokine-rich media, responses of most of the old cases were reconstituted by the addition of conditioned media. On the other hand Mohagheghpourn et al. (1985) reported that defective cell mediated immunity in lepromatous leprosy patients was associated with defective IL-2 receptors and was not reconstituted by the addition of exogenous IL-2. In a subsequent study Mohagheghpourn et al. (1987) showed that in vitro responsiveness of LL patients to *M. leprae* could be restored if the cells were cultured in *M. leprae*-free medium for 48 hours. Presence of *M. leprae* in the preculture medium blocked the reactivity to *M. leprae* in these cultures. They suggest that the apparent specific anergy seen in vivo in LL patients could be due to the persistence of antigen.

IFN-γ plays an important role in activating the macrophages which eventually leads to the killing of microbes residing in these cells. A number of investigators have analysed the production of IFN-γ by T cells from leprosy patients. Noguiera et al. (1983) reported that
Peripheral blood mononuclear cells from lepromatous leprosy patients failed to release IFN-γ in response to *M. leprae*. However, Kaplan *et al.* (1986) suggested that the immune defect in lepromatous leprosy probably results from a lack of response to *M. leprae* by the patients' T cells as they observed that the lepromatous patients responded to *M. leprae* with an enhanced production of H₂O₂ in a fashion similar to that of cells obtained from normal donors.

Ramos *et al.* (1989), have put forward a hypothesis that the lymphokine patterns in LL patients i.e absence of IL-2 and IFN-γ production point to the defective response of the Th1 population, while the enhanced B cell specific-and polyclonal activation as well as generation of cytotoxic (suppressor) cells would be a consequence of overactivation of the Th2 population. In contrast a balanced activation of both populations, in TT, would lead to a successful defence against the mycobacterium. They suggest that three factors could be foreseen to play a determining role in this context a) the MHC-encoded alleles carried by the individual might influence the type of response- generally presentation by MHC class II would lead to the stimulation of Th type of cells and class I molecules would lead to the activation of CTLs; b) alternatively, the MHC antigens present might preferentially associate with a particular mycobacterial determinant, and thus favour the activation of
one or the other of the Th-cell populations; c) the mode of antigen presentation will depend on the route of infection and this will, perhaps, govern the response. Infact, Winters and Humpheres (1990) in a study on mice found that the route of immunization does play a role on the type of immune response generated. They observed that i.d immunization of mice with killed *M. leprae* led to the generation of proliferative responses and these mice were resistant to subsequent challenge with live *M. leprae*. On the other hand i.v immunization induced non-responsiveness and these mice were unable to contain a subsequent challenge with live *M. leprae*.

2.7.2.1.4 Suppressive mechanisms in leprosy

As discussed above, the specific unresponsiveness in LL patients does not seem to be due to lack of *M. leprae*-reactive T cells. Many workers have suggested that the unresponsiveness is brought about by active suppression mediated by suppressor T cells and/or suppressor factors.

Muthukkaruppan *et al.* (1987, 1988) suggested that impaired immune response in LL patients was a result of defective CD2 modulation on the T cells. However, studies carried out by Wong *et al* (1989) demonstrated that CD2 expression was normal on T cells in LL skin lesions and that CD2 expression in PBLs was functional in T cell activation.
2.7.2.1.4.1 Suppressor T cells

Bjune et al. (1979) showed that *M. leprae* antigens caused a generalised suppression of the *in vitro* responses to PHA in all leprosy patients and healthy contacts. Mehra et al. (1979) observed suppression of T cell responses to Con A by Dharmendra lepromin selectively in lepromatous patients but not in tuberculoid patients or healthy contacts. They further showed that TH$_2$+/OK T$_8^+$ subset was involved in suppressing the specific antigenic responses of the LL and BL PBMCs (Mehra et al., 1980). They saw increased levels of Ia and Fc receptors on T cells in LL patients (Mehra et al., 1982) and suggested that these markers may serve as an index of active suppressor activity *in vivo*. In fact, out of 10 *M. leprae*+BCG vaccinated LL patients the number of Ia$^+$T cells in 7/8 patients returned to normal levels along with a marked clinical improvement and conversion to lepromin positivity. Bloom and Mehra (1981) put forth a hypothesis stating that *M. leprae* has one or a small number of unique antigenic determinants capable of recruiting suppressor cells which have the ability to block the responsiveness of helper T cells to other cross-protective determinants. In subsequent studies Mehra et al. (1984) have demonstrated that PGL-1 is capable of inducing suppression in LL patients. They have also correlated their findings with the presence of increased number of T8$^+$ cells in the lepromatous lesions.
and an increased number of T4+ cells in the tuberculoid lesions (van Voorhis et al., 1982, Modlin et al., 1983). In a later study, Modlin et al. (1988) found that in lepromatous lesions, T cells of T-suppressor phenotype (9.3-) were the predominant CD8+ cells and suppressor/inducer cells (2H4+, Leu-8+) represented half of the CD4+ subset. In tuberculoid lesions, helper T cells (CD4+, 4B4+) outnumbered suppressor/inducer cells, 14:1 compared to a ratio of 1.2:1 in peripheral blood.

Nath et al. (1979) also reported suppression of Con A response by M.leprae antigens. However, in contrast to Mehra et al. (1979) they observed this suppression in majority of the tuberculoid patients but only in a few of the LL patients studied. By the 6th day they found suppression across the spectrum. They also found normal levels of suppressor T cell subset as identified by the presence of Fc receptors for IgG (Moretta et al., 1977) in tuberculoid patients and a reduction in their levels in the LL patients (Singh and Nath, 1980). They, therefore, associate the lack of suppressor T cell activity in LL patients with the excessive production of antibodies and autoantibodies. Lack of Ts activity in LL has also been reported by other workers (Stoner et al. 1981, 1982; Bullock et al., 1982; Shannon et al. 1981).

Touw et al. (1980) reported inhibition of proliferative responses of leprosy patients and normals to antigen and mitogen by high concentration of M.leprae. Sengupta et
al. (1987) found a significant inhibition in tuberculin reaction in half of both LL and BT patients when injected along with soluble extract of *M. leprae* (leprosin), the leprosin-derived 12KDa antigen or leprosin depleted of the 12KDa antigen. They did not observe this suppression in healthy controls from endemic region. This finding was not observed in *in vitro* cultures. Kaplan *et al.* 1989 studied the influence of *M. leprae* and its soluble products on the cutaneous responsiveness of leprosy patients to antigen and recombinant IL-2. They concluded that suppressor T cells, if they exist, do not influence the gross or microscopic responsiveness of a cell mediated skin reaction to antigen and IL-2.

Ottenhoff *et al.* (1986) reported that CD8+ Ts clones isolated from a BL patient could specifically suppress responses of peripheral Th cells as well as T cell clones induced by both *M. leprae* and other mycobacteria but not unrelated antigen or mitogen. The suppression was also observed in Th cell responses against a *M. leprae*-specific 36KDa protein which prompted them to suggest the presence of a suppression-inducing determinant on this protein. Modlin *et al.* (1986) showed that T8+ suppressor clones could suppress responses of HLA-D matched but not HLA-D mismatched T4+ clones to *M. leprae* antigens. Salgame *et al.* (1989) have also demonstrated that CD8+ cells isolated from lepromatous leprosy pa-
tients could suppress responses of CD4+ Th clones or PBLs. The suppression required induction by specific M.leprae antigen but was effected in an antigen non-specific fashion. Moreover, while the responses of Th clones were HLA-DR restricted, the Ts cells were able to suppress the response of DR mismatched Th clones as well. More recently Hannen et al. (1990) have shown that M.leprae-specific T cells from a TT leprosy patient were able to suppress HLA-DR3-restricted T cell responses to an immunodominant epitope on the 65K antigen. In this study DR3-restricted responses to an irrelevant antigen such as tetanus toxin were also suppressed by the M.leprae-specific T cell clones. However, non-DR3-restricted responses were not suppressed. Molloy et al. (1990) observed a suppression in mitogenic proliferative response to anti-CD3 when M.leprae sonicate was added to either PBMC or isolated T cells. This non-specific suppression was seen in LL, TT and healthy contacts with purified LAM or M.leprae and M.tuberculosis but deacetylated derivatives had no effect. They suggest that this suppression was due to LPS contamination present in all mycobacterial preparations. Shroff et al. (1990 c) correlated suppression to the organism and route of immunization. They observed that in case of slow growers T cell mediated suppression was only a component of the immune response whereas in case
of rapid growers T cell mediated suppression was the predominant response with the intra peritoneal route of immunization.

While studies described above suggest the involvement of Ts cells, those carried out by Gonzalez-Amaro et al. (1987) ascribed the unresponsiveness in LL to a defective contrasuppressor immune circuit.

2.7.2.1.4.2 Macrophage/monocyte mediated suppression

Suppression of T cell responses by adherent cells, mainly macrophages has also been reported in leprosy. Many workers have suggested that the unresponsiveness associated with lepromatous leprosy is brought about by the production of suppressor factors by monocytes/macrophages of these patients. Sathish et al. (1983) reported the presence of monocyte-derived soluble factor(s) of >25,000 daltons molecular weight in patients with lepromatous leprosy, which suppressed in vitro proliferative responses. Salgame et al. (1983) showed that cell lysates from lepromatous macrophages with specificity for M. leprae could suppress in vitro lymphoproliferation. The effect of factors produced by adherent cells on IL-2 production was studied by Nath et al. (1984a). They reported that monocyte/macrophage derived suppressor factors from borderline and lepromatous patients inhibited the production of IL-2 by Jurkat cells. Factors produced by tuberculoid patients and healthy contacts under similar experimental conditions had little effect
on IL-2 production.

2.7.2.2 Gamma-delta T cells in mycobacterial infections

Most of the work reported till recently was on T cells bearing the αβ T cell receptor (TCR) complex. Lately, however, T cells bearing the γδ receptor have been reported by several groups to be generated in response to mycobacterial infections. Janis et al. (1989) reported an increase in the number of γδ positive T cells as compared to the number of cells bearing αβ TCR, in the draining lymph nodes of mice immunized with *M. tuberculosis*. They did not find any MHC class II restriction for antigen recognition by these cells. O'Brien et al. (1989) generated a series of γδ TCR surface positive hybridomas to investigate the possible function of TCR γδ-expressing lymphocytes. They found that every spontaneously reactive γδ+ hybridoma was stimulated by PPD of *M. tuberculosis*. They suggest that this could be due to cross-reaction with a bacterial antigen. Modlin et al. (1989), found that in contrast to the normal low frequency of γδ TCR-bearing cells in lymphoid tissues, peripheral blood or normal skin, the frequency is increased 5 to eight fold in particular in granulomatous reactions in leprosy. γδ TCR positive lymphocyte lines from leprosy skin lesions proliferate *in vitro* specifically in response to mycobacterial antigens. They found that this reactivity was in the
context of self molecules. They also observed the presence of GM-CSF in the culture supernatants of activated \( \tau \delta \) T lymphocytes. They, therefore, suggested that these cells may be playing a role in the immune response by stimulating granuloma formation. Haregewoin et al. (1989) raised a \( \tau \delta^+ \alpha \beta^- \) CD4^-CD8^- T cell line from a PPD immune individual and observed specific proliferative response of this TCL to PPD and recombinant hsp of relative molecular mass 65 KDa. They observed increased proliferation in the presence of autologous APC. The response decreased in the presence of APC that were matched for only some HLA class I or II antigens. Born et al. (1990) reported murine \( \tau \delta \) T cells recognizing mycobacterial hsp 65. These T cells also responded, though weakly, to murine hsp 63. The antigenic determinant recognized by these cells overlapped with an epitope recognized by arthritogenic \( \alpha \beta \) T cell clones. The data suggested that \( \tau \delta \) T cells might have a role in autoimmune disorders. Studies carried out on \( \tau \delta \) T cells by Rajesekar et al. (1990) also suggest cross antigenecity of mycobacterial heat shock proteins and murine stress proteins. These T cells, the authors suggest could be visualised to play a dual role. On the one hand they could mount a specific immune response against pathogens and on the other hand their reactivity to self stress proteins could allow them to initiate or amplify the immune reactions localised at the site of reaction.
2.8 Genetic influences on immune response to *M. leprae*

Spickett proposed in 1962 that the type of leprosy was determined by hereditary factors. Beiguleman (1972) reported increased concordance for leprosy type within families. Chakravarti and Vogel (1973) studied monozygotic and dizygotic twins concordant or discordant for leprosy type and found that monozygotic twins had a much higher concordance for leprosy as well as leprosy type.

Several studies on class I antigens showed weak associations confined only to the population tested and were usually not reproducible. This implied that class I specificities were not the right markers for HLA encoded Ir/Is genes (Ottenhoff & de Vries, 1987 a & b).

Studies on HLA class II specificities showed more convincing and consistent data. Increased frequency of DR2 in tuberculoid leprosy patients has been reported in studies carried out in Mexicans (Gorodezky et al., 1987), Surinam negroid leprosy patients (Ottenhoff and de Vries, 1987) and Venezuelans (van Eden et al., 1985). However, it appears that genetic mechanisms differ between populations since HLA-DR3 has been found to be associated with tuberculoid disease in Venezuela (Ottenhoff & de Vries, 1987) and Surinam (van Eden et al., 1982).

DQw1 has been found to be associated with lepromatous leprosy in several populations (de Vries et al., 1984;
In summary, it can be concluded that the disease itself is not governed by the HLA linked genes but the type of disease is governed, in part, by these genes.

2.9 Vaccines

Convit (1974) was the first one to develop a vaccine strategy against leprosy. He observed that given alone *M. leprae* persisted as acid fast bacilli for long periods of time in the skin of lepromatous patients. However, when it was inoculated together with live BCG, a granulomatous response was produced to BCG and all acid fast bacilli, including *M. leprae* were rapidly degraded. This suggested that live BCG given along with killed *M. leprae* brings about an immune response to some (common) mycobacterial antigens which leads to the clearance of *M. leprae* from the skin of the usually unresponsive lepromatous patients. Convit and his colleagues (1980) conducted immunotherapeutic trials with live BCG and killed *M. leprae* and observed immunological conversion to skin test positivity, clearance of bacilli from the skin, histopathological upgradation towards the tuberculous pole of the spectrum and clinical improvement in about 75% of BL patients and 57% of polar LLs. The results being encouraging, 3 major vaccine trials were started involving 450,000 people all over the world using this combination. However, even if the vaccine is
effective, the inevitable shortage of *M. leprae* would limit the use of a vaccine based on *M. leprae*, either alone (Shepard, 1976; Patel and Lefford, 1978 and WHO Bulletin) or in combination with BCG. Therefore, there was a need for developing alternate vaccines based on cross-reactive cultivable mycobacteria.

The criteria for such a vaccine are that the organism should be analogous to *M. leprae* in terms of antigens involved in CMI functions. It should also have immunizing potential to induce protective immunity. The obvious choice was BCG which was already being used as a vaccine against tuberculosis. Fernandez in 1939 first injected BCG into healthy lepromin negative children and found over 90% conversion in them. The first experimental evidence in favour of BCG was provided by Shepard (1965, 1966). Analysis of the effect of BCG on incidence of leprosy showed that protection ranged from 20% among 28,000 children in a highly endemic area in Burma to 80% in 16,000 tuberculin-negative children known to have family contact with leprosy. In the south India trial on 210,000 people, BCG showed 30% protection against leprosy. Because of its limited protective value, search was directed towards either an organism or a combination which could elicit better immunity.

In a systematic study with 16 cultivable mycobacteria, five strains *viz.* *M. w, ICRC, M. vaccae, M. phlei* and *M. gordonae* were observed to resemble *M. leprae* in a part
of their antigenic make-up (Mustafa and Talwar, 1978a). Two of them i.e. *M. phlei* and *M. vaccae* had poor immunizing capacity as tested in animals. *M. gordonae* was abandoned because of lack of selective response and strong flare up reactions (Mustafa and Talwar, 1978b). Out of the remaining two, *M. w.* was more potent in its immunogenicity than the ICRC bacillus. ICRC, which is a slow growing mycobacterium belonging to the *M. avium* group is also undergoing clinical trials in Bombay since 1979 (Deo et al., 1981). Gamma-irradiated ICRC induced lepromin conversion in 53% of LL patients and 95% lepromin-negative healthy persons in endemic areas (Deo 1989).

*M. habana* has been reported to bring about protection in the mouse foot-pad model for leprosy and is, therefore, also being proposed as a candidate vaccine (Singh et al., 1985, 1989).

2.9.1 Development of a second generation subunit vaccine

For the development of an effective vaccine against any disease it is essential to identify potentially protective antigens. However, because *M. leprae* has not been cultivated *in vitro* till date, carrying out such studies posed a serious problem in leprosy. With the advent of recombinant DNA technologies it has been possible to construct the genomic library of *M. leprae* and *M. tuberculosis* in λgt11 phage system in *E. coli* (Young et al., 1985 a&b) in plasmid vectors (A626) (Clark-Curtiss
et al. 1985, Khandekar et al., 1986) and in streptomycetes species (Lamb, 1987, Jacobs et al., 1986, Keiser et al., 1986, Cohen et al., 1987). The molecular cloning of M.leprae genes has provided an alternate rich source of supply of protein antigens for immunologic studies as well as for potential vaccine manufacture. Screening of these libraries with the available monoclonal antibodies led to the identification of six major antigens of M.leprae with molecular weights of 12, 18, 28, 36, 65 and 70 KDa. Several of these recombinant antigens have been shown to stimulate T-cell mediated immune response both in vitro and in vivo (Mustafa, 1986; Ottenhoff et al., 1986; Lamb et al., 1987). M.leprae specific T cell clones have been used to directly screen crude phage lysates expressing the proteins to identify T cell activating antigens independent of their recognition by antibodies (Mustafa et al., 1988b; Salgame et al., 1986). The amino acid sequence of a number of T and B cell-activating determinants have been deduced and synthetic peptides have been generated and tested for their ability to induce proliferation of T cell lines and clones from leprosy patients. However, there is a still long way to go before a protective antigen(s) can be identified.

Another approach which has been recently adopted to identify antigens which trigger T cell responses in leprosy is the technique of 'T cell blotting'. The
technique was first reported by Young and Lamb (1986) and later modified by Abou- Zeid et al. in 1987. It involves the separation of antigens on SDS-PAGE followed by transfer onto nitrocellulose. The NC-bound antigens are then directly analysed for their ability to induce T cell proliferation in vitro. A number of studies have already been carried out with fractionated antigens of *M.leprae, M.tuberculosis* and BCG using peripheral blood mononuclear cells from tuberculoid patients, lepromatous patients and healthy contacts of leprosy patients. A number of antigens of interest have been identified using this technique. Young and Lamb (1988) suggest that this technique could be used for identifying suppressor determinants in *M.leprae*. Mehra et al. (1989) have used this technique for the identification of T cell-stimulating antigenic determinants on the cell-wall of *M.leprae*.

2.9.2 Other candidate vaccines

Vermani and Mahadevan (1986) and Marolia and Mahadevan (1987) have reported that the delipidified portion of the insoluble components of *M.leprae* (DCC) act as an immunomodulatory agent. Recently Marolia and Mahadevan (1990) reported that DCC can stimulate immune-deficient cells of leprosy patients through Reactive oxygen intermediate formation. Because of its potential immunomodulatory nature the DCC, of *M.leprae* it could be used
as a vaccine. Gelber et al. (1990) also reported that immunisation of mice with *Mycobacterium leprae* derived cell wall fractions, progressively depleted of lipids, carbohydrates and soluble proteins derived from the pellet of *M. leprae* sonicate conferred protection against *M. leprae* challenge.

In another study Mehra et al. (1989) using precursor frequency analysis, observed that a very large proportion of T cell clones reactive with intact *M. leprae* were stimulated by highly purified cell walls of *M. leprae*. This cell wall component induced DTH reaction in guinea pigs and human volunteers.

Chirmule et al. (1989) reported the fractionation of ICRC bacillus to identify its immunogenic components. They obtained a very high molecular weight component (approx. 1000KDa) named PP-I, which is a dominant immunogen. A similar fraction has been isolated from *M. leprae* and the PP-I fractions of the two organisms show antigenic cross-reactivity. A vaccine containing PP-I of ICRC induced lepromin conversion in 30% of LL patients and their lepromin negative household contacts (Bhatki et al., 1989).

2.9.3 *Mycobacterium w*

*Mycobacterium w* is an atypical, non-pathogenic, rapid growing mycobacterium first isolated from the sputum of a tuberculosis patient. It is niacin negative and positive for semi-quantitative catalase and heat resistant
catalase. It lacks the ability to hydrolyse Tween-80 upto 10 days but degrades sodium salicylate. It reduces nitrate to nitrite. It grows on Lowenstein-Jensen medium and Middlebrook medium. It does not form pigment either in the dark or in light. On the basis of its growth characteristics, it is classified in Runyon's group IV. However, it differs from the other strains previously included in this group (Saxena et al., 1978; Katoch, 1981).

In earlier studies (Mustafa and Talwar, 1978a) M.w was observed to induce blast transformation and lymphocyte migration inhibition in peripheral blood lymphocytes of TT patients. It also induced cross-reactive skin responses with M.leprae in guinea pigs (Mustafa and Talwar, 1978b), gave strong DTH reactions in mice (Fotedar et al., 1978) and caused enlargement of draining lymph nodes in mice (Mustafa and Talwar, 1978c). It produced Dharmendra and Mitsuda reactions similar to M.leprae in TT patients and unlike lepromin it evoked positive responses in LL patients also (Mustafa and Talwar, 1978d; Hogerzeil et al., 1978; Girdhar and Desikan, 1978; Govil and Bhutani, 1978).

Intradermal immunization with M.w converted a large percentage of lepromin negative LL/BL patients to lepromin positivity (Chaudhuri et al., 1983). Based on all these studies M.w was selected as a candidate anti-leprosy vaccine. After extensive toxicology
studies and approval by the Drug regulatory authorities a vaccine based on killed M.w is currently undergoing 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. In addition to clinical improvement the vaccine brings about histopathological and immunological improvement as evidenced by faster bacterial clearance, and lepromin conversion. Initial studies have shown 61.1% to 75% lepromin conversion in lepromin negative LL patients. 100% of the BB and 85.7-100% of the BL also showed lepromin positivity following vaccination (Talwar et al., 1990a, 1990b).

In recent studies Mustafa (1988) demonstrated that M.w has antigens that cross-react with at least three major protein antigens of M.leprae. Ganju et all. (1990) have demonstrated the presence of M.leprae and M.tuberculosis cross-reactive B cell determinants on M.w using anti-M.leprae and anti-M.tuberculosis monoclonal antibodies as well as leprosy and tuberculosis patient sera.