CHAPTER 2

REVIEW OF LITERATURE
2.1 History
2.2 Spectrum of the disease
2.3 Immunology of leprosy
  2.3.1 Cell mediated immune (CMI) response
  2.3.2 Humoral immune response
2.4 Immunodiagnostic methods
  2.4.1 Immunoassays based on antibody detection
  2.4.2 Immunoassays based on antigen detection
2.5 Vaccines for leprosy
2.6 Features of nerve damage in leprosy
  2.6.1 Predilection sites
  2.6.2 Mechanisms of nerve damage
  2.6.3 Host-parasite interaction
2.7 Experimental models for leprosy
  2.7.1 Animal models
  2.7.2 Nerve tissue culture model
2.8 Cell mediated immune response during nerve damage
  2.8.1 Cytokines
  2.8.2 Humoral immune response
Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. It affects mainly the peripheral nerves. It also affects the skin, muscles, eyes, bones, testes and internal organs. The disease is clinically characterised by cardinal features such as total loss of sensation in the affected areas, presence of thickened nerves and presence of acid-fast bacilli in the skin smears.

2.1 History

Leprosy is probably the oldest disease known to mankind. For a long time, the disease was confused with psoriasis, elephantiasis and pellagra. In India, the disease is known since ancient times as 'Kustha roga' or 'maha roga' and attributed to punishment or curse from God. During the middle ages, leprosy was widespread in almost all countries in the world. Thereafter, it declined slowly in many European countries, partly due to strict isolation and partly due to improvement in the standard of living and quality of life of the people.

Modern day leprosy dates from 1873 when Hansen of Norway discovered *M. leprae*. For long years, there was no effective remedy for leprosy. The introduction of sulphone drugs in the treatment of leprosy in 1943 marked the beginning of a new era - the Era of case finding and domiciliary treatment. Leprosy has since become a curable and controllable disease.
Fig. 1. The spectrum of leprosy
### 2.2 Spectrum of the Disease

Clinical symptoms and signs in leprosy are extremely varied, with respect both to nature and extent. Danielsen and Boeck (1847) classified leprosy into two types - nodular and anaesthetic. However, a more differentiated and detailed classification was given by Ridley & Jopling (1966). They classified leprosy patients into five categories and in 1974 Ridley updated and enlarged the classification into a six group system (1974, 1977) (Table I and Fig.1).

**TT:** In these patients, very few lesions are seen. These are often macular or raised usually with loss of pigmentation, sweating and tactile sensitivity. Occasionally patients come with only one thickened nerve. Histopathologically, the TT lesion is dominated by heavy infiltration of lymphocytes around "clouds" of epithelioid cells. Very few or no acid-fast bacilli (AFB) are seen.

**BT:** The skin lesions resemble those of TT leprosy, but are usually smaller and multiple. Larger nerves are frequently found to be enlarged and less commonly cutaneous sensory nerves. Histopathologically, the lesions resemble those of TT leprosy, but there are fewer lymphocytes and the granuloma does not usually extend into the epidermis. Few AFB's are detected.

**BB:** The patients usually have numerous skin lesions which may be either erythematous or hypopigmented. The lesions have a symmetrical pattern and widespread enlargement of nerves is often found. Histopathologically, the lesions consist of epithelioid cell granulomas, but in contrast to TT and BT
<table>
<thead>
<tr>
<th>Table I</th>
<th>The Leprosy Spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>Polar tuberculoid</td>
</tr>
<tr>
<td>BT</td>
<td>borderline tuberculoid</td>
</tr>
<tr>
<td>BB</td>
<td>borderline</td>
</tr>
<tr>
<td>BL</td>
<td>borderline lepromatous</td>
</tr>
<tr>
<td>LL</td>
<td>lepromatous</td>
</tr>
<tr>
<td></td>
<td>LLs (subpolar lepromatous)</td>
</tr>
<tr>
<td></td>
<td>LLP (polar lepromatous)</td>
</tr>
</tbody>
</table>
lesions, no giant cells are found. The lymphocytes in the lesions are relatively few, and they are found in a diffuse pattern throughout the granuloma. AFB's are regularly detectable.

BL: The skin lesions are numerous, consisting of erythematous or hyperpigmented papules, nodules or plaques or ill-defined hypopigmented macules. Histopathologically, BL lesions consist of undifferentiated macrophages or histiocytes, usually without epithelioid cells. The macrophages may also show degenerative changes. Lymphocytes are often scanty, but may occasionally be found in larger numbers extending throughout the granuloma. Numerous AFB are usually found in these lesions.

LL: Skin lesions are very numerous, consisting of small hypopigmented macules or small papules. The skin is diffusely infiltrated and thickened, and enlarged ear lobules are frequently seen. Other changes of the face include thinning of eyebrows and lashes, and destruction of nasal cartilage and bone. As the disease progresses, diffuse bilateral and generally symmetrical nerve damage occurs, leading to progressive anesthesia. AFB's are found in both skin and nerve lesions.

2.3 Immunology of leprosy
The spectral concept of leprosy is quite useful in considering the immunological aspects of this disease. An inverse relation between the intensity of delayed hypersensitivity and so called "serologic activity" in
response to *M.leprae* is seen throughout the clinical spectrum.

Cellular immune responses to *M.leprae* have been tested throughout the Ridley and Jopling scale both by lymphocyte transformation (LTT) and leukocyte migration inhibition (LMIT) tests as well as delayed skin reactivity to *M.leprae*, and continuous decrease in activity from the tuberculoid towards the lepromatous end of the scale has been found (Myrvang et al. 1973 a & b). The presence of antimycobacterial antibodies in serum, on the other hand, increased towards the lepromatous end (Godal, 1974). These antibodies were detected by ordinary double gel-diffusion technique using disintegrated *M.duvalii*, as *M.leprae* was not available in sufficiently large quantities at that time. However, more recently established radioimmunoassays and the fluorescent antibody test of Abe et al. ,(1976) suggest that a considerable proportion of BT patients may also have increased levels of antimycobacterial antibodies (Huber et al. 1976; Harboe et al. 1977) including antibodies specific for *M.leprae* (Abe,1973; Abe, et al. 1976; Harboe et al. 1977).

Taken together, both delayed hypersensitivity reaction in vivo and the in vitro lymphocyte responses to *M.leprae* suggest that clinical manifestations in leprosy to a large extent are determined by the capacity of each subject to mount a cell-mediated immune response to *M.leprae*. Further support for this conclusion comes from studies on leprous lymph nodes (Turk and Waters, 1971), and experimental
studies in mice. Turk and Waters (1971) described a shift in the appearance of paracortical (thymus-dependent) areas from being well developed in tuberculoid cases to being extensively replaced with parasitized macrophages in lepromatous cases. While normal DBA mice develop microscopic lesions with histological characteristics of BB-BT lesions (Rees et al. 1969), mice immunologically suppressed by X-ray irradiation and thymectomy (Rees et al. 1967) or thymectomy and antilymphocyte serum (Gaugas, 1968) develop lesions which mimic lepromatous leprosy. The close association found both clinically and experimentally between epithelioid cell formation and thymus cell function may suggest that epithelioid cells in leprosy represent T cell activated macrophages or a more inactive end-stage of the activation process, analogous to the plasma cell in the B cell system (Godal, 1978).

2.3.1 Cell mediated immune (CMI) response
CMI is mediated by T lymphocytes and macrophages. There may be a generalized defect in CMI or the defect may be restricted to a failure in recognising a specific antigen. In leprosy, earlier studies have shown that LL patients are comparatively less responsive to in vivo stimulation on contact sensitization with picryl chloride and dinitrochloro benzene (Waldorf et al. 1966; Bullock, 1968). A number of studies have been made with mitogens to find out the lymphoproliferative response in patients (Dierks and Shepard, 1968; Sheagren et al. 1969; Bullock and Fasal, 1971). The
observation of lowered mitogenic response in LL patients was unequivocal. Effective chemotherapy reduces the bacillary load in these patients and the mitogenic response gets restored. However, there is no evidence that this state of generalised depression makes LL patients susceptible to other infections like leishmaniasis and tetanus.

CMI is the result of a complex interplay between the macrophages engaged in processing the antigen and T cells in recognising the antigen. Hence all studies have been carried out with macrophages and T cells obtained from LL and TT patients and normal healthy individuals. The first observation that macrophages from LL patients are incapable of killing *M. leprae* (Beiguelman, 1967) was contradicted by Godal and Rees (1970). While working on the capability of *M. leprae* antigen presentation by the macrophages from leprosy patients, Hirschberg (1978) noted that macrophages of LL patients were unable to present *M. leprae* antigens to T lymphocytes. However, Nath et al. (1980) and Stoner et al. (1982) failed to observe such a defect in a HLA-D matched situation. Moreover, macrophages from LL patients were found to secrete normal levels of IL-1 when stimulated with *M. leprae*. Further, when investigated for Ia expression Narayanan et al. (1983) could not find any difference in the Ia expression between TT and LL patients.

Various in vivo and in vitro studies that have been carried out indicate that the defect in CMI in leprosy is primarily *M. leprae* specific. While TT patients are able to mount a positive in vivo lepromin reaction and can show lymphocyte
positive in vivo lepromin reaction and can show lymphocyte blastogenic response to \textit{M.leprae}, LL patients fail to do so (Mitsuda, 1919; Rees, 1964; Bullock and Fasal, 1971; Godal et al. 1971; Myrvang et al. 1973 a & b; Myrvang, 1974). Nevertheless LL patients show good CMI to other mycobacterial preparations/antigens like PPD and BCG. Such data strongly suggests that the state of unresponsiveness in LL is due to an anergy specific to \textit{M.leprae} only.

Since early seventies, T-cell defect has been implicated in LL. In 1971, Godal et al. stated that \textit{M.leprae} reactive T cells are absent in LL patients. Recently Haregewoin et al. (1983) and Nath et al. (1984) have shown that there is no clonal deletion of T-cells in LL. T cells in LL patients were found incapable of proliferation due to the deficiency of IL-2. It was further noted that IL-2 was essential for induction of lymphocytes of LL patients to produce IFN-$\gamma$ in response to \textit{M.leprae} (Nogueira et al. 1983). However, reversibility of in vitro response of LL patients by exogenous supply of IL-2 was not unequivocal. Such detailed studies of the functions of different cellular components of CMI suggest a T cell defect in recognising \textit{M.leprae} antigen(s) in LL patients.

\textit{BCG} with \textit{M.leprae} has been shown to activate the macrophages of LL patients in vivo. This activation ultimately led to the clearance of \textit{M.leprae} from the lesions (Convit et al. 1979). Recently other mycobacterial species like ICRC (Deo et al. 1981) and \textit{M.w} (Chaudhuri et al. 1983, Talwar et al. 1990) have also been found to be capable of clearing \textit{M.leprae}
from LL patients. All these studies indicate that even in LL, if macrophages are properly activated they are able to kill *M. leprae*. Kaplan et al. (1986) showed that the monocytes of LL patients activated *in vitro* by IFN-γ generate more H₂O₂ than unactivated monocytes.

Another mechanism which may bring about the defect in CMI is suppression of T cell function by some factor liberated by macrophages (Salgame et al. 1983) or by antigens of *M. leprae* as shown *in vivo* (Sengupta et al. 1984) or by generation of suppressor T cells (Mehra et al. 1979, 1980). *In vivo* studies carried out with T cell phenotypic markers in granulomas of leprosy patients showed that while in TT granulomas the helper suppressor cell ratio is 2:1, the ratio is reversed in LL granulomas (Narayanan, 1988). Narayanan et al. (1988) further demonstrated the presence of a cytotoxic factor in the supernatants of LL granuloma.

2.3.2. Humoral immune response:

Humoral immune response appears to be unimpaired in leprosy. Patients usually have raised levels of immunoglobulins and are capable of forming antibodies to bacterial vaccines such as typhoid or small pox vaccines (Jha et al. 1971; Saha et al. 1973). Numerous workers have reported the levels of immunoglobulins in leprosy sera using radial immuno-diffusion methods (Lim and Fusaro, 1968; Sheagren et al. 1969; Bullock et al. 1970; Malaviya et al. 1972; Saha and Mittal, 1972; Srisinha et al. 1972; Graboz et al. 1973; Srivastava et al. 1975; Kelkar et al. 1979; Sengupta et al. 1979; Chaudhary et
A correlation has been reported by several workers between the type of leprosy and the class of immunoglobulins and sometimes contradictory reports have been made. Sengupta et al. (1979) found IgG to be at a significantly higher level in all types of leprosy and in household contacts of lepromatous cases as compared to controls. However, IgA antibody level was significantly higher than normal controls in all types of leprosy except tuberculoid, while IgM was raised only in borderline tuberculoid (BT) patients. The data indicates that there is some antigenic stimulation without manifestation of any disease. This was confirmed by Abe et al. (1976), who demonstrated specific anti-\textit{M.leprae} antibodies in sera of contacts of leprosy cases by fluorescent assay. Lim and Fusaro (1968) observed that IgG, IgA and IgM levels were significantly increased in lepromatous patients. Similar finding was observed by Chaudhary et al. (1987). Saha and Mittal (1972) noted increase in IgA and IgM levels in lepromatous patients, while Sheagren et al. (1969) found that serum IgG and IgA antibody levels in such cases. Kelkar et al (1979) found that serum IgG was markedly raised in both lepromatous and tuberculoid patients, whereas the increase in IgM and IgA type of antibodies was not statistically significant when compared to that of normal individuals. Although leprosy patients develop antibodies against specific as well as cross-reacting antigens of \textit{M.leprae}, it is believed that humoral immunity does not alter the course of leprosy infection. It may indeed be harmful as the
antibodies react with *M. leprae* antigens in the tissues during type 2 lepra reaction (ENL reaction) resulting in the deposition of immune complexes in the damaged tissues. Besides antibodies against the leprosy bacilli, there appear to be present autoantibodies against a variety of self antigens e.g. nucleus, mitochondria, thyroid, microsomes, smooth muscles and DNA (Bonomo et al. 1963; Mathews and Trautman, 1965; Bonomo and Dammacco, 1971; Malaviya et al. 1972; Shwe, 1972; Petchlai and Chuttanondhi, 1973; Wright, 1973; Wall and Wright, 1974; Saha et al. 1975; Rea et al. 1976; Yumnam et al. 1977; Linder et al. 1979; Furukawa et al. 1984; George et al. 1986). Appearance of autoantibodies in TT patients suggest that factors other than depressed cell mediated immunity are involved in autoantibody production in leprosy (Masala et al. 1979).

Presence of antigen-antibody complexes has been demonstrated in leprosy, tuberculosis and in various other diseases by several workers using different methods (Affronti, 1959; Adam et al. 1972; Moran et al. 1972; Axelsson et al. 1973; Audibert et al. 1976; Bjorvatn et al. 1976; Saha and Chakrabarty, 1977; Lambert et al. 1978; Furukawa et al. 1984; Ramanathan et al. 1984). It has also been described in healthy subjects and in physiological conditions such as pregnancy (Mason, 1977). It has been suggested that circulating immune complexes (CIC) may be involved in the pathogenesis of erythema nodosum leprosum reaction (Ridley and Jopling, 1966; Wemambu et al. 1969; Moran et. al 1972; Lambert et al. 1978).
Ramanathan et al. (1984) found that circulating immune complexes (CIC) from TT patients consisted largely of IgG and C3. Leprosy sera have been studied for the presence of the CIC by a number of methods and majority of the samples screened were positive for CIC (Benjamin and Daniel, 1982). Using methods based on complement mediated and receptor mediated binding techniques it has been shown that while complement mediated complexes were more marked in lepromatous and reactional leprosy, receptor mediated complexes were predominant in tuberculoid leprosy (Geniteau et al. 1981). Other studies using platelet aggregation tests indicated that CIC were present in a large percentage of cases of leprosy, but there was no diagnostic or prognostic significance in relation to the disease (Sehgal and Kumar, 1981).

Paca de Azevedo and Homen de Melo (1966) reported that the complement activity of sera of LL patients in lepra reaction was decreased as compared to LL without reaction and TT patients. On the other hand, Sheagren et al. (1967), showed distinctly increased complement activity in LL patients. Tausk et al. (1985) have reported that the number of C3b receptors on erythrocytes of LL patients was significantly decreased when compared to the normal individuals or tuberculoid patients.

2.4 Immunodiagnostic methods

Leprosy is a major health problem in developing countries. Despite the existence of apparently effective chemotherapy since the 1940’s, the prevalence of leprosy in many endemic
countries has remained high.
One of the reasons for this slow or no decline in prevalence rate, incidence rate, is due to lack of availability of simple, reliable and sensitive test for the detection of the whole spectrum of the disease. Further, since the incubation period of the disease may range anything from 2-15 years, there is need to have sensitive and specific diagnostic tests for early diagnosis of leprosy.
A number of immunoassays for diagnosis of leprosy have been reported. The titration of anti-mycobacterial antibodies in patient sera, has been the basis of most of these assays. Recently, detection of \textit{M. leprae} antigens in serum, urine and tissues has also been the subject of study.

2.4.1 \textbf{Immunoassays based on antibody detection}

The earliest serological test, the FLA-ABS (Fluorescent leprosy antibody-absorption) test, was proposed by Abe et al. in 1976. The test is based on detection of \textit{M. leprae} antibodies in leprosy sera. The percentage positivity for LL varied from 94.7 to 99.3 in comparison to 76.5 to 86.1 for TT. However, the patient serum needs absorption with cardiolipin, lecithin and polysaccharide of tubercle bacilli, and sonicated suspension of BCG and \textit{M. vaccae} to remove antibodies directed against cross-reactive antigens of \textit{M. leprae}.

Harboe et al. (1978) reported a RIA (radioimmunoassay) using radio-labeled \textit{M. leprae} filtrate as the antigen. The assay was specific for leprosy, however, absorption of patient
serum with BCG sonicate is required to render the assay specific.

A number of enzyme immunoassays (EIA's) for immunodiagnosis of leprosy have been described. Of these the assays based on phenolic glycolipid I (PGL-I) are highly specific for leprosy. PGL-I (Hunter and Brennan, 1981) is an antigen unique to \textit{M. leprae}, and has a trisaccharide as the immunodeterminant. The terminal disaccharide and the monosaccharide residues of PGL-I have been chemically synthesized (Gigg et al. 1983; Fujiwara et al. 1984). The assays based on PGL-I or synthetic sugar residues are quite sensitive for detection of active lepromatous patients with high bacillary load. In several independent studies with PGL-I (Cho et al. 1983, 1984; Brennan, 1983; Young and Buchanan, 1983; Ralhan et al. 1985; Bach et al. 1986; Meeker et al. 1986; Gonzalez & Gonzalez, 1987), the percent positivity for LL patients ranged from 58.4 to 100 in contrast to 35 to 57 for TT patients. In assays using the synthetic disaccharide (Cho et al. 1984; Fujiwara et al. 1984; Brett et al. 1986; Sanchez et al. 1986) the positivity for multibacillary (LL/BL) patients varied from 74 to 97 percent in comparison to 28 to 61.54 percent for paucibacillary (TT/BT) patients. Though the assays were highly specific for leprosy, a false positivity with tuberculosis sera has been reported. Young et al. (1985a) have reported a "spot" test using PGL-I as the antigen and polysulphone membrane as the solid phase.
EIA's using *M. smegmatis*, either intact cells (Douglas et al. 1984) or arabinomannan (Miller et al. 1983) have been employed for serodiagnosis of leprosy as well as for monitoring of chemotherapy. Sera of LL patients showed high reactivity with *M. smegmatis*. With arabinomannan purified from *M. smegmatis* the ability of the assay to diagnose LL patients was 95 percent, but for TT patient it was only 27 per cent. The assay is not specific for leprosy. An EIA using *M. fortuitum* as the antigen has also been reported (Vithayasai et al. 1983). The patient serum needed absorption with *M. marinum* to render the assay specific for leprosy. Sera from patients with bacilliferous leprosy gave high reactivity, but there was overlap with values of normal sera.

The availability of *M. leprae* specific monoclonal antibodies (MoAbs) has provided a new thrust to the development of these immunoassays. Sinha et al. (1985) reported a serum antibody competitive test (SACT) using a MoAb (MLO4) directed against My2a determinant of *M. leprae*. The assay is based on inhibition of binding of $^{125}$I labeled MLO4 antibodies to *M. leprae* sonicate by antibodies in leprosy sera. The assay is highly specific for leprosy. Sera from normal healthy individuals, and patients with pulmonary tuberculosis or diseases like cancer and autoimmune diseases were negative in the assay. Seropositivity was 100 per cent in multibacillary and 91 percent in paucibacillary patients. False positivity with tuberculosis sera was 8.8 per cent.

By and large, most of the antibody based assays described
above, are quite sensitive for detection of multibacillary patients and the utility of these assays for diagnosis of paucibacillary leprosy is fairly low.

2.4.2 Immunoassays based on antigen detection
Detection of *M. leprae* antigens in the clinical specimen (serum, urine etc.) would be better indicators of infection. Young et al. (1985b) have reported the detection of PGL-I in serum and urine of LL and BL patients. The detection limit of the assay was about 50 ng PGL-I per ml of serum. It was observed that level of circulating antigen decreased shortly after initiation of therapy. Similar results were also reported by Cho et al. in 1986, but in their ELISA, sensitivity was high (500pg). 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). Olcen et al. (1983, 1986) have also reported the detection of *M. leprae* antigens in the urine of LL patients, by an inhibitory RIA. A significant correlation between bacterial index (BI) and antigen concentration was found. The detection limit of the antigen was 20 ug. The amount of antigen excreted in urine decreased with effective chemotherapy.

2.5 Vaccines for leprosy
Various approaches have been employed to develop a vaccine against leprosy. The main problem is that no experimental model is available which simulates exactly the human
lepromatous leprosy, the form against which a vaccine is ideally required. Furthermore, the latent period of the disease is several years and this serves as a big damper in the evaluation of a potential immunoprophylactic agent against the disease. Notwithstanding these limitations to-date four vaccines have been proposed as under:

<table>
<thead>
<tr>
<th>Name &amp; Type</th>
<th>Research Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG</td>
<td>Fernandez (1939)</td>
</tr>
<tr>
<td>BCG &amp; M.vaccae</td>
<td>Stanford et al. (1981)</td>
</tr>
<tr>
<td>Killed ICRC</td>
<td>Deo et al. (1981)</td>
</tr>
<tr>
<td>Killed M.w</td>
<td>Talwar (1978)</td>
</tr>
</tbody>
</table>

Although *M. leprae* was a logical candidate for immunization as it has been shown to confer immunity in mice against itself (Shepard et al. 1976; Patel and Lefford, 1978) or in combination with BCG (Stanford, 1978), there were some important limitations in its use. Being a pathogen, it cannot be administered in a live form. Experience with other vaccines has shown that for long lasting immunity and with a high CMI response, live vaccines fare much better than those based on killed organisms. For this reason, feasibility of utilizing a cultivable non-pathogenic mycobacteria was explored. The bacteria should be analogous to *M. leprae* in terms of antigens involved in CMI functions. It should also have immunizing potential to induce protective immunity. Five mycobacteria 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 reactions (Mustafa and Talwar, 1978b). Out of the remaining two, *M. w* was more potent in its immunogenicity than the ICRC bacillus. *M. w* induced cross-reactive skin reactions with *M. leprae* in guinea pigs (Mustafa and Talwar, 1978b), showed strong, delayed type hypersensitivity (DTH) response in mice (Fotedar et al. 1978) and enlarged draining lymph nodes in mice (Mustafa and Talwar, 1978c). It produced Dharmendra and Mitsuda reactions similar to *M. leprae* lepromin in tuberculoid leprosy patients, while it differed from *M. leprae* in also evoking positive reactions in lepromatous leprosy patients.

Recently, application of recombinant DNA technology and availability of *M. leprae* specific monoclonal antibodies (MoAbs) have made it possible to identify and characterise at least six protein antigens from *M. leprae* which could have a potential to be used as subunit vaccine.

Genomic libraries have been made by cloning mycobacterial DNA into *E. coli* (Young et al. 1985a & b; Clark-Curtiss et al. 1985). Subsequent screening of the libraries using MoAbs (Engers et al. 1985), led to the identification of partial antigens. Several of these recombinant antigens have been shown to stimulate T-cell mediated immune response both in *vitro* and *in vivo* (Mustafa, 1986; Ottenhoff, 1986; Lamb et al. 1988). The characterization of recombinant *M. leprae* antigens for their ability to trigger appropriate CMI responses via T-cells represents a major effort of many laboratories world-
wide. Simultaneously, purified cell wall protein (peptidoglycan-protein complex) has been found to have the ability to elicit all the immunological properties of *M. leprae* (Robinson and Mahadevan, 1987). Some of the cell wall protein antigens have been isolated and found to possess the capacity to induce cell mediated immune response (Vermani and Mahadevan, 1988; Hunter et al. 1989; Mehra et al. 1989).

2.6 Features of Nerve Damage in Leprosy

Leprosy with its global population of over 15 million patients, constitutes the largest single disorder of the peripheral nervous system considering the involvement of intradermal nerves, other sensory nerves, the mixed nerve trunks and the intramuscular nerves, in all types of the disease.

Leprosy differs in several features viz: clinical, immunological, histopathological as well as neuropathological all along the disease spectrum. Abundant investigative work has been carried out to understand the neuropathology of leprosy (Nishiura, 1960; Iyer, 1965; Antia et al. 1975; Job and Verghese, 1975; Weddell and Pearson, 1975; Pedley et al. 1980; Shetty et al. 1980 a,b & c; Srinivasan et al. 1982). Some studies have shown concordance between skin and nerve pathology (Pedley et al. 1980) while report to the contrary also exist (Srinivasan et al. 1982). The histopathological features of nerve involvement in leprosy have been particularly well reviewed by Weddell and Pearson (1975) according to whom, though there is good correlation between
the histopathologic and clinical features of the developed disease, and one can be deduced from the other, when one tries to fit nerve damage into this picture, there is no clear cut range of findings. Certain generalisations can be made: for instance, marked nerve trunk enlargement is more common in non-lepromatous than in lepromatous leprosy, the skin lesions of tuberculoid leprosy are always anesthetic, denoting that dermal nerve damage is a constant feature of this type of disease, multiple nerve trunk damage tends to be an early feature of the mid-spectrum borderline (dimorphous) type of leprosy. However there are many exceptions. Gross nerve trunk enlargement and damage can often be found in lepromatous leprosy. Also in advanced lepromatous leprosy there can be severe dermal nerve damage. Clearly, the degree of nerve damage manifested in leprosy is not directly and solely related to the degree of host resistance, and is not susceptible to neat classification along these lines. There are, however, basic differences between the ways in which the nerves are damaged in the two "polar" forms of leprosy, characterized by the typical epithelioid and small mononuclear cells accompanied by the paucity of acid fast organisms in tuberculoid leprosy on the one hand, and by the large foamy bacteria laden macrophage (the lepra cell) of the lepromatous variety on the other. The nerve damage in tuberculoid leprosy is more severe in onset and faster in progression, but is more localised. The damage in tuberculoid leprosy is due to the infiltrating epithelioid
and mononuclear cells which effectively combat the bacillary infection but cause severe nerve damage in the process. The cellular infiltrate destroys the morphological structure completely many times giving rise to caseation which is ultimately replaced by fibrotic materials. The subtler but more extensive spread of the infection in lepromatous leprosy is due to the heavy loads of bacilli which disrupt the normal functioning of Schwann cells, perineural cells etc. thus giving rise to demyelination, proliferation of perineural and endoneural cells which ultimately destroy the nerve and induce collagenisation of the tissue. Thus the end result in extreme cases of infection at both the polar end of leprosy are collagenisation of the nerve and irreversible damage. However, while in the initial stages of tuberculoid nerve damage, the onset is acute and fast, the nerve damage in lepromatous leprosy is diffuse, gradual, and in the initial stages the nerve structure is well preserved probably due to the absence of cellular infiltrate.

2.6.1 Predilection sites
Though in leprosy, parasitization of variety of cells occurs (Kaur et al. 1975), the host cell for the majority of M.leprae is either the macrophage or the Schwann cell. This predilection for the Schwann cells of the peripheral nerves is a unique feature of this disease and has been described by various workers (Iyer, 1965; Job and Verghese, 1975). There are indications that the Schwann cell serves not only as the natural host for the growth and multiplication of M.leprae
but also protects the organism from the body's immune responses as well as from drugs (Ridley, 1973; Antia and Kamala, 1983).

These observations have further led to the speculation that the Schwann cell resident \textit{M. leprae} may possibly be a primary and persistent source of infection for the continuous leakage of bacilli or bacillary antigen into the circulation, hence responsible for the state of persistent infection or relapse seen in some lepromatous cases (Waters et al. 1978; Stoner, 1979). Peripheral nerve damage is therefore a universal phenomenon across the spectrum of leprosy, with at least 25% of the patients suffering from deformities and sensory impairment and in extreme cases motor impairment. According to Stoner (1979) nerve involvement in leprosy may represent an essential phase in the cycle of infection and reinfection by \textit{M. leprae} responsible for the bacillary dissemination and the suppression of cell-mediated immunity, and this unique characteristic of neural affinity, which can be so debilitating to the host, very likely confers a significant advantage on the ability of the bacillus to survive as a species.

It is therefore evident that with the Schwann cell emerging as the primary target for \textit{M. leprae} (Antia, 1982), the study of the host-parasite relationship between \textit{M. leprae} and Schwann cells is of prime importance. An \textit{in vitro} tissue culture model has been established by Mukherjee et al. (1980 a & b) which has enabled the study of this relationship directly at the cellular and molecular level.
The *in vitro* model has proved useful in confirming the histopathological and electronmicroscopic observations made *in vivo* in human and experimental leprosy and has given further insight to the direct interaction between *M. leprae* and components of nerve, particularly the Schwann cell. That the Schwann cells are the target for invasion and dissemination of *M. leprae* has been demonstrated *in vitro* by comparing the relative ability of *M. leprae* to parasitize Schwann cells, skin fibroblasts and muscle cells (Sangle et al. 1984).

It was also shown that adherence and phagocytosis of other mycobacteria by Schwann cells was poor as compared to that of *M. leprae* (Mukherjee and Antia, 1985a). The adherence and phagocytosis of *M. leprae* is seen to be an active process requiring the host and the parasite to be metabolically active and is mediated by the recognition of surface molecules by receptors on the host cell (Itty et al. 1986). *M. leprae* phagocytosis by Schwann cells is seen to differ from that of macrophages with the involvement of receptors other than those found on the macrophages. Cytomorphologically no detrimental effect on the Schwann cells can be seen on their parasitization with *M. leprae*. Only the specialized functions of the Schwann cells like DNA synthesis, proliferation, migration and association with axons are affected (Mukherjee and Antia, 1985 b & c). Besides these host cell modifications, whether *M. leprae* interaction gives rise to the secretion of factors (as is evident in the macrophage
M.leprae interaction) and thus modified the behavior of the host Schwann cell, particularly its interactions with other cells involved in cell mediated immunity, to its advantage is still to be elucidated.

The mechanism(s) by which Schwann cell resident M.leprae evade the host microbicidal action and immune surveillance is not clear. At the lepromatous end, where the peripheral nerves and Schwann cells are heavily loaded with bacilli, but are not associated with the lymphocytic infiltrate, the damage is difficult to explain.

2.6.2 Mechanisms of Nerve Damage

Since the most striking aspect of leprosy is the tragic deformity resulting from untreated infection, it was long believed that damage to nerves was caused by the invasion of nerve cells by the bacilli. M.leprae grows primarily in two cells of the body, mononuclear phagocytes and Schwann cells surrounding peripheral nerve.

Wisniewski and Bloom in 1975 and Cammer et al. in 1978 have through a guinea pig model tried to understand how nerves could be damaged in tuberculoid leprosy. Briefly guinea pigs or rabbits were sensitized to foreign antigens in complete Freund’s adjuvant using killed mycobacteria. When such animals were tested with PPD (purified protein derivative of tuberculin), a delayed-type hypersensitivity reaction was produced in the tissues. Although this represented a specific cell-mediated immune response to a non-neural antigen, there was invariably primary focal demyelination
observed to the local nerves, which were damaged as innocent bystanders. This has been termed as "bystander" demyelination.

Recent findings suggest that cytotoxic T lymphocytes from tuberculoid leprosy patients can lyse *M. leprae* infected Schwann cells, suggesting a second mechanism of immunological damage to nerves (Steinhoff and Kaufmann, 1988). The mechanisms of nerve damage in lepromatous leprosy may also be immunological. In this circumstance, damage occurs to small peripheral sensory nerves, in the absence of white cells infiltration. A major clue as to the possible mechanism underlying this form of nerve damage was provided by the finding of highly elevated levels of C3d, a breakdown product of the major complement component in the blood of lepromatous patients (Bjorvatn et al. 1976). Since lepromatous patients have elevated levels of antibodies to *M. leprae* antigens, the model for tissue damage suggested would be release of mycobacterial antigens in the tissues, for example, spontaneously or perhaps following, chemotherapy, leading to local antibody-antigen reactions in the tissues in the vicinity of the small sensory nerve fibers. The fixation of complement and release of pharmacologically active complement components and mediators would lead to permeability change and edema. This sequence of events is likely to produce compression neuropathy that would irreversibly damage the small sensory nerve fibers.

Many critical questions relating to factors involved and the possible mechanisms in nerve damage in leprosy, e.g. the
mechanism of entry and dissemination of *M. leprae*; the interaction between the parasite and the various neural constituents or the type and pattern of degeneration etc. still remain unanswered.

2.6.3 Host-parasite interaction

The Schwann cell emerges as one of the prime target cells for *M. leprae*. A host of clinical, histopathological, electron microscopic studies carried out in human as well as experimental leprosy *in vivo* and *in vitro*, all point to the unique affinity between *M. leprae* and Schwann cells (Rees et al. 1963; Weddell et al. 1963; Lumsden, 1964; Iyer, 1965; Job and Verghese, 1975; Lalitha et al. 1977, Antia, 1982; Mukherjee and Antia, 1986). The Schwann cell serves not only as the natural host for the growth and multiplication of *M. leprae* but also protects the organisms from the body’s immune responses as well as from drugs (Ridley, 1973; Antia and Kamala, 1983). These observations have further led to the speculation that the Schwann cell resident *M. leprae* may possibly be a primary and persistent source of infection for the continuous leakage of bacilli or bacillary antigen into the circulation, hence responsible for the state of persistent infection or relapse seen in some lepromatous cases (Waters et al. 1978; Stoner, 1979).

Both the Schwann cells and the macrophages phagocytose *M. leprae* avidly. Nevertheless it must be kept in mind that Schwann cell is not a professional phagocyte as the macrophage; its main function being myelin synthesis and so
its phagocytic characteristics are quite distinct from that reported for macrophages (Varon and Manthorpe, 1982; Mukherjee et al. 1980a; Silverstein and Loike, 1980).

The phagocytosis of *M. leprae* by Schwann cells is slow, the optimum phagocytosis being only 72 hours after inoculation with the bacilli (Mukherjee et al. 1980a) as compared to the faster period of phagocytosis of the macrophages. Whereas the macrophages avidly take up viable as well as dead bacilli, the phagocytosis by Schwann cells is significantly reduced by heat-killing or formal-saline treatment of *M. leprae* (Mukherjee et al. 1980a & b) or drastic purification procedures to remove host tissue contamination (Unpublished observations quoted by Mukherjee and Antia, 1986). The phagocytosis is blocked by lipase and enhanced by trypsin (Itty, M.Sc thesis 1985, Itty et al. 1986). It is also significantly inhibited by agents that cause metabolic inhibitions or disruptions of cytoskeletal elements of the host Schwann cells (Itty, M.Sc Thesis, 1985) and is also influenced by the state of association of the Schwann cell with axon (Mukherjee and Antia, 1985c).

Some of these factors may possibly have contributed to the reported 'no ingestion' or poor ingestion of *M. leprae* in the system of Fildes (1974) and Saito et al. (1986). Phagocytosis of other mycobacteria by Schwann cell has been reported to be poor (Mukherjee and Antia, 1986).

It is well established in macrophages that the phagocytosis of mycobacteria, including *M. leprae* is mediated
by the receptors for the Fc portion of the immunoglobulin G (Bar-Shavit et al. 1979; Unkeless, 1980) as well as for the C3B portion of complement (Bianco et al. 1975; Griffin et al. 1975). More recently lectin receptors have also been implicated (Weir et al. 1982; Sharon, 1984). There is no experimental demonstration of the existence of any of these receptors in the mouse Schwann cell membrane. Preliminary experiments carried out by Mukherjee and Antia (1986, Unpublished observation) did not reveal the presence of Fc or C3b receptors on these cells. The absence of Fc receptor in the cultured rat Schwann cells has also been reported by Mirsky (1980). Phagocytosis of M. leprae by the Schwann cell is mediated by other receptor(s), possibly lipid in nature, and not either monosaccharide or protein residue(s) (Itty, M.Sc. Thesis, 1985). Further experiments directed towards isolation and characterization of receptors on Schwann cell membrane may lead to a better understanding of the molecular basis for the ability of M. leprae to invade Schwann cells. As already observed in the interaction between M. leprae and macrophages, the mycobacterium has the ability to modify some of the host cells functions e.g. M. leprae infection of macrophages altered macrophage membranes reducing the Fc receptor expression (Birdi et al. 1983) and also caused the release of suppressor factor(s) which affected protein synthesis and transformation of lymphocytes (Salgame et al. 1980). It also altered the lipid metabolism of the macrophages (Kurup and Mahadevan, 1982).

Similarly Schwann cells on infection with M. leprae underwent
certain alterations. The properties of attachment, migration and proliferation of Schwann cells were impaired on long term infection with \textit{M. leprae} (Mukherjee and Antia, 1985c). However, there appeared to be no cytopathic changes or host cell lysis during the course of infection (Mukherjee and Antia, 1985b). The rate of protein synthesis, too, remained unaltered (Mukherjee and Antia, 1980b).

Thus, the \textit{in vitro} studies conducted on the Schwann cell-\textit{M. leprae} interaction demonstrate the nontoxic effect of \textit{M. leprae} on the peripheral nerves, and suggest that the infected cells may be unable to participate in the regenerative activity of the nerves but continue to survive for a prolonged period of time. This phenomenon may, however, initiate slow but progressive nerve damage and also contribute to the spread of bacilli within the nerve as well as in the systemic circulation. These effects can, to an extent, be reversed by prolonged chemotherapy (Mukherjee and Antia, 1985c). Similar observations have also been made in histopathological studies of lepromatous nerves where poor proliferation as well as poor axon association were observed (Job and Verghese, 1975).

Besides this, it is also evident that though \textit{M. leprae} parasitized both macrophages and Schwann cells, often to the same extent, its interaction with the two host cells is vastly different.

It now remains to be seen whether the \textit{M. leprae} Schwann cell interaction gives rise to the generation of factor(s)
which affect the external milieu, like the suppressor factor(s) of *M. leprae* infected macrophages (Salgame et al. 1980).

2.7 Experimental models for leprosy

2.7.1 Animal models

The decades of 1960's and 1970's witnessed great strides in the development of experimental models. In 1960, Shepard discovered that *M. leprae* could multiply to a limited extent when injected into foot pads of mice. Multiplication of *M. leprae* occurs in a similar if not identical fashion to that in normal mice when the organisms are inoculated into immunologically normal rats (Fieldsteel and McIntosh, 1971; Hilson 1965), hamsters (Shepard, 1960 a & b; Waters and Petit, 1965), gerbils (Shepard, 1960 a & b) and mystromys (Binford, 1968). Inoculation either systemically or locally, of immunosuppressed or immunodeficient rodents with *M. leprae* results in a greater maximum of multiplication. Most of the published work has involved the adult-thymectomized, lethally-irradiated and bone-marrow reconstituted (T900R) mouse originally employed by Rees (1966) and confirmed by Gaugas (1967). Because it has appeared that the degree of immunosuppression required to permit unlimited multiplication of *M. leprae* is incompatible with long survival of the rodent species, investigators had to turn to the specific-pathogen-free or germ-free congenitally-athymic "nude" mouse. Colston and Hilson (1976) and Kohsaka et al. (1978) suggested greater enhancement of the multiplication of *M. leprae* in these
animals than in T900R or neonatally-thymectomized Lewis rat. Inoculation with *M. leprae* of the nine-banded armadillo (Dasypus novemcinctus Linn), initially undertaken because of the low core temperature (30-36°C) characteristic of this animal species (Storrs, 1974), has resulted in progressive leprosy like disease of a large proportion of the animals (Amescua et al. 1979; Kirchheimer and Storrs, 1971).

Armadillos have a life span of 12 to 14 years. This is far in excess of ordinary mice (2 years) or even mystromys (5 years). Dasypus novemcinctus, Linn. has unique potential for leprosy research because it regularly produces monozygous quadruplets, making it possible to replicate experiments with genetically identical animals. This is particularly relevant to leprosy research because of the genetic basis of the assumed mechanism of resistance of human beings to leprosy (Spickett, 1964). In more recent research, Wolf et al. in 1983 discussed successful transmission to rhesus and African green monkeys using intravenous and intradermal inoculation. Discovery of naturally occurring leprosy in a mangabey monkey (Meyers et al. 1980, 1985) sparked interest in that species potential and successful transmissions were reported in 1984 (Martin et al. 1984; Meyers et al. 1984) and 1985 (Martin et al. 1985; Wolf et al. 1985). Baskin et al. (1987) reported successful induction of disseminated leprosy in African green monkeys by inoculating them intravenously and intracutaneously with *M. leprae* derived from a naturally infected mangabey monkey. Some of these monkeys developed signs and symptoms of nerve damage (Waters et al. 1978; Wolf
Subsequently, there were reports that African green monkeys inoculated intravenously with *M. leprae* developed active leprosy infection in peripheral nerves with extensive inflammation and with polyneuritic features (Baskin et al. 1987).

2.7.2 Nerve tissue culture model

Harrison, in 1907 conducted a series of classic experiments and described the nerve tissue culture technique, which was later modified and developed by Murray and Stout (1940, 1942). The advantage of this system are: it provides tissues in a steady functional state, and also, cellular components of the sensory ganglia and nerves can be visualised directly. This technique would thus help in studying directly the interactions between *M. leprae* and the Schwann cells and also the changes in the Schwann cell membrane properties on infection with *M. leprae*. Two types of *in vitro* cultures were developed viz: explant or organ cultures (Murray and Kopeck, 1953; Murray, 1965; Mukherjee et al. 1980a & b) and dissociated monolayer cell cultures (Varon, 1970, 1975; Itty et al. 1986).

The light microscopic morphology of the Schwann cells are long slender spindle shaped bipolar cells with a large oval nucleus and one or two nucleoli. Schwann cells in dissociated cultures of new born rat sciatic nerve can be identified by Ran-1 which is a surface antigen marker for rat Schwann cells (Brockes et al. 1977). Mouse Schwann cells in

The Schwann cell is of particular interest in this connection, for although M.leprae are frequently seen in these cells (Weddell et al. 1963), the mode of their entry into them is unknown. Therefore, it is important to study this interaction between the Schwann cells and M.leprae.

2.8 Cell mediated immune response

M.leprae has been found within the nerve structures in all the leprosy forms, however, the bacillary load does not seem to be the direct cause for the nerve lesion (Hamida et al. 1987). An overwhelming majority of the endoneural bacilli are found in the Schwann cell cytoplasm, but M.leprae can also be found in endothelial cells and fibroblasts localized within the endoneurium (Dastur et al. 1973). The segmental demyelination frequently observed could be the result of the Schwann cell destruction, but this destruction is not a constant finding and preserved Schwann cells are seen covering demyelinated fibres with evidence of axon damage or even without axon damage (Hamida et al. 1987). In the tuberculoid form of the disease, even in the areas where granulomatous lesions are not present, there is active myelin destruction in association with the presence of some inflammatory cells (Nilsen et al. 1986). Schwann cells could be involved in the induction of
antigen-specific T-cell mediated immune response to *M. leprae* derived determinants in the peripheral nerves. These cells have been demonstrated to function as antigen presenting cells in a class II restricted manner (Wekerle et al. 1987; Samuel et al. 1987). It is noteworthy that immunohistochemical studies have detected, in nerves from lepromatous patients, lymphocytes and macrophages intermingled with Schwann cells expressing HLA-DR antigens (Nilsen et al. 1986). Murine *M. leprae*-specific CD8+ T lymphocytes are able to kill by antigen-specific cytotoxic activity; *M. leprae* infected Schwann cells in vitro, expressing class I MHC antigens induced in the presence of IFN-γ (Steinhoff and Kaufmann, 1988).

Some experimental observations give support to the hypothesis that the nerve lesions in leprosy are mediated by cytokines. A direct myelinolytic effect of TNF-α was observed when myelinated cultures of murine spinal cord were grown in the presence of this mediator, but not with IFN-γ or IL-2 (Selmaj and Raine, 1988). Lymph node cells from mice primed with myelin basic protein, when left in culture for 48 hours, synthesize a soluble factor, probably TNF-α that induces myelin breakdown in vitro (Watson et al. 1989).

Antigen-specific T-cell mediated hypersensitivity to *M. leprae* antigens is associated with the pathogenesis of major nerve trunk damage in the tuberculoid forms of leprosy. This T-cell dependent mechanism is observed more frequently during acute reversal reactions (Shegal et al., 1988). But the
mechanism of nerve damage in the lepromatous forms of the disease is totally unknown. The serum level of the inflammatory cytokines (TNF-α, IL-1 and IL-6) have been observed to range from detectable to very high levels in the lepromatous form of leprosy, but not in the tuberculoid form (Sarno et al. 1991). The production of inflammatory cytokines during ENL episode could be a consequence of the exacerbation of T-cell independent mechanisms operational at the tissue level even in the absence of reaction. But it can be postulated that during the course of the ENL episode, the response pattern of the lepromatous patient to *M. leprae* antigens is modified. Analysis of blood leukocytes in ENL patient demonstrate increase in the CD4+/CD8+ ratio (Rao and Rao 1987; Modlin et al. 1985), higher frequency of IL-2 receptor-expressing cells and emergence of *M. leprae* reactive T-cells (Rea et al. 1984; Laal et al. 1985). In the serum of ENL patient macrophage inhibitory activity factor (MIF) and elevated levels of soluble IL-2 receptor were observed in contrast with the lack of MIF activity and lower levels of the receptor observed in absence of reaction, in the lepromatous patients (Tung et al. 1987). As a consequence of the modification in the cellular immunity during ENL, it can also be postulated that during the reaction episode, the activation of mononuclear phagocytes could occur in response to a previously ineffective or absent T-cell mediated pathway. The evaluation of parameters of mononuclear phagocyte activation will provide evidence for the occurrence of this mononuclear phagocyte activation. The determination
of the presence of the cytokines and of the cytokine specific mRNA's in the reactional lesion, will help in assessing the involvement of these mediators in the inflammatory process.

2.8.1 Cytokines

When immune cells are stimulated in a variety of ways, they release soluble proteins called cytokines. Because of their far-reaching effects on many different types of cells and tissues, cytokines can be considered hormones. Among the cytokines, interferon and the interleukins are well established as important mediators of the inflammatory response (Zeigler, 1988). Cytokines are essential transmitters of cell-to-cell communication in many physiological and pathophysiological processes. Recent reports suggest that these plurifunctional proteins modulate or mediate many essential biological processes, primarily those concerned with cell growth and differentiation (Le and Vilcek, 1987). Two major monocyte-macrophage derived cytokines, tumor necrosis factor (TNF) and interleukin 1 (IL-1) have been well characterized. The other cytokines being IL-2, IFN-γ, IL-4, IL-6, IL-10 and GM-CSF.

Studies by Haregewoin et al. (1983) have shown that although lepromatous T cells fail to produce IL-2 after exposure to *M. leprae* they can proliferate in response to *M. leprae* in the presence of T-cell conditioned medium, suggesting that the unresponsiveness in lepromatous leprosy results from a deficiency in the production of IL-2 or
related factors and not a lack of *M. leprae*-reactive T-cells. Recent evidence has implicated IFN-γ as the important macrophage-activating factor in lymphokine preparations in both tumoricidal and microbicidal systems (Pace et al. 1983; Wisseman and Waddell 1983; Nathan et al. 1983). Nogueira et al. (1983) reported that the peripheral blood lymphocytes of patients with lepromatous leprosy failed to produce IFN-γ upon exposure to *M. leprae* and showed reduced responses to Con A, but this deficiency was restored on addition of purified human IL-2.

Several cytokines have been shown to regulate the expression of specific isotypes by affecting either the frequency of isotype switching or allowing maturation of precommitted precursors. IL-4 enhances the production of IgG1 and is required for IgE production in vitro and in vivo. IFN-γ was shown to stimulate the secretion of IgG2a in concentrations at which it inhibits IL-4 induced production of IgG1 and IgE. IL-5 on the other hand, appears to stimulate maturation (Coffman et al. 1989). Kindler et al. (1989) reported that injection of rabbit anti-TNF antibody, after 1 or 2 weeks of infection, dramatically interferes with the development of BCG-induced granulomas (both in number and size, large epithelioid cells failing to appear) and subsequent mycobacterial elimination. There are speculations that cytokines are released during reactional stages of leprosy - possibly TNF-α and IL-1. TNF is the secretory product of monocytes/macrophages (Beutler and Cerami, 1986), large granular lymphocytes
(Peters et al. 1986) and activated PBL (Cuturi et al. 1987). It is reported to have several bioactivities like activation of neutrophils (Shaalaby et al. 1985; Gambie et al. 1985; Klebanoff et al. 1986), potentiation of coagulation (Nawroth et al. 1986), stimulation of resorption of bone (Bertoloni et al. 1986) and cartilages (Sakalatvala, 1986), induction of secretion of IL-1 (Libby et al. 1986), GM-CSF (Munker et al. 1986) and PGE$_2$ (Dayer et al. 1985). It is also suspected to be one of the inflammatory and pathological mediators in septic shock (Tracey et al. 1987; Waage et al. 1987) and cerebral malaria (Grau et al. 1989). Likewise, IL-1 is known to be produced by a wide variety of cell types and to exert pleiotropic effects (Dinarello and Savage, 1989). TNF, IL-1 and IL-6 are the products of different genes that encode nonhomologous proteins and bind distinct receptors. Despite this, there is a considerable overlap in the cellular sources and the biological activities of the three cytokines (Dinarello, 1989; Beutler and Cerami, 1988; van Snick, 1990; Mizel, 1989; Heinrich et al. 1990). TNF and IL-1 can induce the biosynthesis of themselves and each other and of IL-6 and the three cytokines often act synergistically e.g. IL-1 and IL-6 synergise in the induction of T-cell activation (Mizel, 1989; van Snick, 1990). This interdependence must be borne in mind when interpreting the effects of one of these cytokines in a biological system. There is evidence that in in vitro tissue culture, TNF is able to directly cause destruction of myelin and oligodendrocytes (Selmaj and Raine,
Such an effect is not due to immune mediated mechanisms, but due to alteration in ion channels of oligodendrocytes as well as axons. Similarly it has potent effect on bone resorption and collagen synthesis in osteoblast like cells in vitro (Thomson et al. 1987; Canalis, 1987). If these observations are extended to leprosy, then it could explain some of the nerve damage and deformities in those cases where there is no direct evidence of granuloma formation. More recent evidence suggests that although there is no organised granuloma or evidence of activation of macrophages in the leprosy lesions; the antigen trapped in lymph nodes could induce local activation of helper T cells as well as APC within the nodes (Desai et al. 1988). High levels of TNF-$\alpha$ and IL-1 have been reported in LL patients (Parida, et al. under publication). The high levels of TNF could be a result of activated T helper cells and macrophages. The patients having high levels of TNF-$\alpha$, also had high levels of IL-1 suggesting the synergistic action of the two cytokines in the biological activity and immunopathological process. The possibility that glial cells of the nervous system may interact with the immune system in the initiation or augmentation of T-cell mediated immune responses has been raised by recent studies (Kingston et al. 1989). Schwann cells do not express major histocompatibility complex (MHC) class II antigen under normal culture conditions. The above studies show that in the presence of sensitized T lymphocytes and antigens, Schwann cells do not require pretreatment with exogenous
IFN-γ to express MHC Class II antigens and function as antigen-presenting cells. T-cell derived TNF and IFN-γ appear to act as mediators of the T-cell induced expression of MHC class II by Schwann cells. Overall, the in vitro results strongly support the view that Schwann cells can participate in neuroimmunological responses involving T lymphocytes and may suggest a possible role for Schwann cells as accessory cells initiating a mechanism of nerve damage in a disease such as leprosy.

2.8.2 Humoral immune response

So far data pertaining to the role of humoral immune response in the induction of nerve damage in leprosy are not available. It is well established that in the LL forms there is significantly high levels of circulating antimycobacterial antibodies (Harboe et al. 1981; Melsom et al. 1982). There are also reports demonstrating the presence of autoantibodies directed against self proteins such as thyroglobulin, rheumatoid factor, C-reactive protein and antinuclear factors (Mathews and Trautman, 1965; Bonomo et al. 1969; Malaviya et al. 1982; Shwe, 1972; Petchclai and Chuttanondhi, 1973; Wright, 1973; Rea et al. 1976; Sengupta et al. 1979; Kano et al. 1981; Touw et al. 1982; Rawlinson et al. 1987), mainly in LL groups of patients. However, there are only few studies reporting the presence of antibodies directed against the peripheral nerves. Wright et al. (1975) reported the presence of antibodies directed against axons of the peripheral nerves. The anti-axonal
antibody occurred in 40% and 20% of lepromatous and tuberculoid leprosy patients respectively. They have also reported that this antibody does not cross-react with M. leprae and that it is not related to nerve damage as it has also been observed in 15% of control sera tested. Another report by Eustis-Turf et al. (1986) showed the presence of antibodies directed against Po protein of PNS myelin and against the intermediate filament proteins. Their explanation to these findings is that leprosy patients produce antibodies to these sequestered nerve proteins which are released subsequent to the bacterial invasion of the peripheral nerves. A companion paper by Benjamins et al. (1989) further characterized the specific antigens. The leprosy sera bound to 35 kDa and 42 kDa molecular weight proteins of intermediate filament. They suggest that the presence of these antibodies alone is insufficient for nerve damage as these antibodies were also detected in a small sample of healthy donors.

In the above studies, antibodies to specific antigens such as axons, Po protein of myelin basic protein and intermediate filaments were looked at. The results in these reports varied in the sense that the presence of these antibodies were not evident in all the patients. It remains yet to be established whether in leprosy, the antibodies are directed to more than one neural antigen. One would expect such a phenomenon as the morphological studies both at light and ultrastructure level show that the destruction is not
limited to any particular component but several of PNS component such as myelin, Schwann cell, axon and perineural cells (Rees et al. 1963; Imaeda and Con vit, 1963; Dastur et al. 1970; Job 1971; Antia, 1982).

Simultaneously a histochemical study has suggested a greater role for B cells in the immunopathology of leprosy than assumed earlier (Kreisler et al. 1973). Antibodies directed against myelin basic protein, cerebrosides, gangliosides and myelin associated glycoprotein has been reported in other peripheral neuropathies such as encephalomyelitis and polyneuritis (Hemachudha et al. 1987). It is possible that such antibodies may also be present in leprosy. This calls for a detailed study of the role of humoral immune response in the pathogenesis of nerve damage in leprosy.