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A. LEPROSY - THE DISEASE

Leprosy is rarely fatal but it has propensity to cause crippling mutilations if left untreated. Extensive deformities and disabilities seen in untreated leprosy patients led people in the past to their social rejection and being treated as pariahs. The intensity of the social stigma associated with the disease is, however, on the decline now. For this, credit goes to availability of various curative measures against the disease which has been made possible by the dexterity and ingenuity of the researchers worldwide in the last two decades.

In developing countries, however, leprosy continues to pose a serious problem. Approximately, 1320 million people live in 28 countries with a registered prevalence of over 1 case per 1000 persons and at a significant risk of contracting the disease (Noordeen, 1991).

Already 5.5 million people are afflicted by leprosy (Noordeen et al., 1992). This number of affected individuals may increase considerably as *M. leprae* is progressively becoming resistant to sulfoines (Ridley, 1983). The data from India also suggests that 7 to 8 years of multidrug therapy (MDT) has failed to fulfil the expected target of lowering the disease incidence (Gupte, 1993). This is one of the reasons which has prompted a number of research groups to look for alternatives such as, vaccines. But outcome of vaccination programme will remain unpredictable until the mechanisms underlying the clinical and immunological spectrum observed in leprosy are completely understood.

Leprosy (Greek: Lepros-scaly, scabby, rough) also called Hansen’s disease is a chronic, non-suppurative, inflammatory condition caused by infection with a
gram positive, acid fast bacillus, *Mycobacterium leprae* (Hansen, 1874). An immunocompetent host presumably handles very efficiently the putative infection by this pathogen and develops no clinical sign of the disease. However, in susceptible hosts immune responses to *M. leprae* are precipitated in the form of granulomatous lesions of skin and nerves, since *M. leprae* is known to have predilection for the peripheral nerves and skin (Ridley, 1971). An order was imposed upon the variety of granulomatous response in different hosts by Ridley and Jopling (Ridley and Jopling, 1966), where a variety of granulomatous postures found in different hosts depended on their degree of resistance to the replication of *M. leprae* inside their tissues. This widely used classification is based upon clinical and histological features paralleled by order of resistance to *M. leprae* infection or cell mediated immunity in patients with leprosy.

Patients with leprosy are classified in different categories (Jordon, 1991) (Table 1):

a) highly resistant tuberculoid pole (TT)
b) borderline tuberculoid (BT)
c) dimorphic or borderline (BB)
d) borderline lepromatous (BL)
e) lepromatous (LL) - low resistant pole with subpolar and polar types.

Patients with tuberculoid form of the disease exhibit exaggerated immunity which results in successful elimination of *M. leprae* from their tissues but generally at the cost of self tissue destruction while patients with the lepromatous form of the disease develop pathological immunity which fails to check exuberant
Table 1: Some clinical, histological and immunological features of the Ridley-Jopling classification of leprosy

<table>
<thead>
<tr>
<th>Clinical, Histological and Immunological Features</th>
<th>Polar Tuberculous (TT)</th>
<th>Borderline with Tuberculous Features (BT)</th>
<th>Borderline (BB)</th>
<th>Borderline with Lepromatous Features (BL)</th>
<th>Polar Lepromatous (LL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin Lesions</td>
<td>Few in number, sharply defined plaques with tendency for central clearing, asymmetrical</td>
<td>Smaller, more numerous than lesions of TT</td>
<td>intermediarily between BT and BL</td>
<td>&quot;Inverted saucer&quot; characteristic but not common. LL type nodules, ill defined plaque with an occasional sharp margins</td>
<td>ill defined nodules, generalised diffuse infiltrate, or xanthoma like papules, symmetrical, leonine facies and eyebrow alopecia</td>
</tr>
<tr>
<td>Nerve Lesions</td>
<td>Skin lesion anesthesia early, nerve trunk palsies</td>
<td>As in TT</td>
<td>As in TT</td>
<td>Mixed TT and LL</td>
<td>Skin lesions, no anesthesia early, nerve trunk palsies variable, symmetrical distal anesthesia</td>
</tr>
<tr>
<td>Lepromin skin test</td>
<td>Positive</td>
<td>Usually positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Histology organisms</td>
<td>Rare less than 1 per 100 oil emersion field</td>
<td>Rare to 1 per 10 oil emersion field</td>
<td>1 to 10 per oil emersion field</td>
<td>10 to 100 per oil emersion field</td>
<td>10 to 1000 per oil immersion field</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Present, dense peripheral infiltration about granuloma; infiltration into epidermis</td>
<td>Present, peripheral infiltration about granuloma, variable epidermal infiltration</td>
<td>Typically lymphoprogenic</td>
<td>Present, moderately dense and in the same distribution as macrophage</td>
<td>Scant, diffuse or in focal distribution</td>
</tr>
<tr>
<td>Macrophage differentiation</td>
<td>Epitheloid</td>
<td>Epitheloid</td>
<td>Epitheloid</td>
<td>Usually undifferentiated, epitheloid foci may be present, may show foamy change</td>
<td>Foamy, may be undifferentiated in early lesions</td>
</tr>
<tr>
<td>Langerhans giant cells</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Immunoperoxidase Studies</td>
<td>2</td>
<td>2</td>
<td>Not studied</td>
<td>0.5-2.0 usually low</td>
<td>0.5</td>
</tr>
<tr>
<td>Lymphocytes Helper: Suppressor Ratio</td>
<td>As in BT</td>
<td>Restricted to the lymphoblastic mantle about epitheloid tubercle</td>
<td>--</td>
<td>Usually as in LL; rarely as BT</td>
<td>Admixed with Macrophages</td>
</tr>
<tr>
<td>Suppressor: Cytotoxic phenotype</td>
<td>As in BT</td>
<td>Admixed with epitheloid cells and in lymphocytic mantle</td>
<td>--</td>
<td>As in LL</td>
<td>Admixed with Macrophages</td>
</tr>
<tr>
<td>Helper: inducer phenotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Heavy Staining in all Categories</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Less in TT than in LL</td>
<td>Variable results reported</td>
<td>Variable results reported</td>
<td>Variable results reported</td>
<td>Variable results reported</td>
</tr>
<tr>
<td>Keratinocytes</td>
<td>--</td>
<td>Strongly expressed</td>
<td>Usually absent</td>
<td>Unusual</td>
<td>Heavy staining</td>
</tr>
<tr>
<td>Langerhans Cells</td>
<td>--</td>
<td>Increased number</td>
<td>Some increase</td>
<td>Some increase</td>
<td>Some increase but usually normal</td>
</tr>
<tr>
<td>Interleukin 2 positive cells</td>
<td>--</td>
<td>1 in 200 cells stain positively</td>
<td>--</td>
<td>1 in 3000 cells stain positively</td>
<td>--</td>
</tr>
</tbody>
</table>
growth of *M. leprae* in their tissues. In some patients pathological lesions may be categorized as indeterminate leprosy. The indeterminate lesions with one or more ill defined hypopigmented macules or patches, occasional acid fast bacilli, perivascular lymphohistocytic infiltrates may regress simultaneously or progress to manifestate a well defined nature or remain indeterminate over a prolonged period of time. Probably indeterminate leprosy occurs prior to the immunologic commitment or determination by the host to self cure or to the development of an overt granulomatous expression of the illness (Khanolkar, 1964). Cell mediated immune responses to *M. leprae* cannot be detected in these patients (Myrvang *et al.*, 1973), although humoral responses may be present in some (Abe *et al.*, 1976).

Leprosy patients also suffer from acute episodic inflammatory reactions during the natural course of the disease (Ridley, 1969). Mostly these reactions fall into:

a) **Type 1 Reversal reactions**: Type 1 reactions reflect heterogeneous immunological phenomenon related to T cells (Laal *et al.*, 1987). Most of responder borderline tuberculoid patients show a deterioration in T cell function during reactions whereas multibacillary nonresponder borderline border and borderline lepromatous patients show improvement in *in vitro* T cell reactivity (Barnetson, 1976).

b) **Type 2 Erythema nodosum leprosum (ENL)**: These reactions occur in over half of lepromatous patients, especially in the course of antileprosy treatment. The appearance of erythema nodules is often associated with fever and is sometimes complicated by neuritis, orchitis, iridocyclitis etc.
Histologically, erythematous dermal nodules show increase in T cells with helper phenotypes (Modlin et al., 1983).

c) Borderline reactions or Lepra reactions: These reactions are associated with severe neuritis and are further subdivided into: i) reversal reactions associated with movement toward the tuberculoid pole; ii) downgrading reactions associated with movement toward the lepromatous pole.

The mechanism leading to such reactions are far from clear. Moreover, attempts to unravel the enigma of leprosy as to why the vast majority of people exposed to infection do not develop clinical disease in the first place and those who do and become lepromatous remain anergic to *M. leprae*, have so far failed to yield conclusive answers.

B. DEFECTS IN HOST CELL MEDIATED IMMUNITY (CMI)

In the classical view, T cell mediated immunity mounted by an immunocompetent host is always seen as a cascade of events which ultimately results in elimination of the invading organisms or antigens. Phagocytes present in peripheral blood or tissues engulf and process any foreign antigen such as invading bacilli and their soluble products. The processed antigens are presented by the antigen presenting cells (APC), macrophages, Langerhan's cells, dendritic cells' to the specific receptors present on T cells in the context of human leukocyte antigen (HLA) molecules. CD4+ helper T cells see an antigen in association with HLA class II molecules and CD8+ cytotoxic /suppresor T cells recognise an antigen in association with class I molecules. If activated, these T
cells produce Interleukin-2 molecule, IL-2 which amplifies proliferation of antigen sensitized cells to enhance the magnitude of immune response against the pathogen. The clonally expanded T cell population release other effector molecules such as Interferon-gamma IFN-γ and tumor necrosis factor alpha-TNF-α which in turn activate macrophages or other phagocytes to facilitate elimination of intracellular pathogens through various bactericidal mechanisms. These cellular interactions mediated via cell surface adhesion molecules and cytokines result in release of IL-1, IL-3, IL-4, IL-8 and other cytokines which influence the function of other immune cells(Sengupta,1993).

A voluminous work has been carried out to unravel the mysteries shrouded by leprosy. Several situations have been envisaged wherein various components playing critical role in cell mediated immunity could behave erroneously, since it has been concluded that cellular immune response mounted by patients with leprosy do not follow the conventional route.

Both lymphocytes and macrophages play the axial roles in the pivotal process of immune response. In leprosy also, these are found to be the major cell populations in lesions from the patients. The macrophages of patients with tuberculoid leprosy show very few bacilli whereas in the lepromatous form of the disease the macrophage bathe in *M. leprae*. Attempts to explore the potential defects which lead to heavy infiltration of bacilli in macrophages and in vivo anergy to *M. leprae* in patients with lepromatous leprosy, focuss largely on determination of efficacy of macrophage mediated functions such as antigen phagocytosis, killing, processing and presentation.
Impaired Antigen Adherence / Phagocytosis / Processing and Presentation

Reports indicating low level of adherence of *M. leprae*, altered membrane topography, downregulation of various membrane bound receptors such as Fc receptors, Con A receptors and HLA-DR antigen expression were suggestive of a membranous defect in lepromatous macrophages (Lad *et al.*, 1983; Birdi *et al.*, 1983). However, the phagocytic abilities of lepromatous macrophages were found to be similar to those from healthy individuals (Oscar Rojas Espinosa, 1978). Therefore, it remained elusive whether these reported membrane perturbations in lepromatous macrophages were of primary importance for the course of infection or a consequence of the development of infection. The metabolic changes subsequent to the phagocytosis were found not to be significantly different, as assessed by levels of various cytoplasmic enzymes, in macrophages either between leprosy patients and controls or between lepromatous and tuberculoid leprosy patients (Avita and Convit, 1970). However, this observation was negated by a report indicating a decrease in some of the hydrolytic enzymes such as lysozymes, lactate dehydrogenase, 8 glucuronidase and leucine uptake in the lepromatous macrophages (Birdi *et al.*, 1979; Marolia and Mahadevan, 1984). A hypothesis based on the idea that the lysosomal enzymes, though present in sufficient amount do not reach the *M. leprae* encased in phagosome, was supported by an observation demonstrating resistance of fusion with lysosomes by a majority of phagolysosomes harbouring freshly isolated, viable *M. leprae* in resident peritoneal macrophages from Swiss Webster mice (Sibley *et al.*, 1987a). The presumptive role played by inherent abilities of *M. leprae* to evade the bactericidal mechanisms in lepromatous macrophages, also has been studied extensively. Two compounds, phenolic glycolipid and superoxide dismutase which are present in *M. leprae*, are
known scavengers of free radicals (Wheeler and Gregory, 1980; Neill and Klebanoff, 1988). It was presumed that these compounds possessed by *M. leprae* can scavenge various reactive oxygen intermediates released by activated macrophages thereby preventing the lysis of bacilli. This was partly supported by an observation indicating lesser generation of superoxide ions and hydrogen peroxide by macrophages of leprosy patients (Marolia and Mahadevan, 1984; 1988). Though, unchecked growth of bacilli in lepromatous tissues could have been explained by this observation, successful elimination of bacilli from normal and tuberculoid macrophages despite the presence of free radical scavengers in *M. leprae*, remained unexplained. Moreover, in long term treated bacillary negative lepromatous leprosy patients’ macrophages, *M. leprae* still remained metabolically active, though sufficient amount of hydrogen peroxide was reported to be present in macrophages. On the other hand, the paucibacillary patient’s macrophages, reported to produce lesser amount of hydrogen peroxide as compared to that by normal macrophage, were able to kill *M. leprae* (Marolia and Mahadevan, 1990). The earlier observations indicating lack of the intrinsic ability to cause *in vitro* lysis of *M. leprae* by macrophages derived from lepromatous patients (Barbieri and Correa, 1967) could not be confirmed by others (Godal and Rees, 1970). When *M. leprae* isolated from tuberculoid and lepromatous macrophages were injected into mouse foot pad, characteristic bacillar growth patterns were observed. However, activation of macrophages from both these groups with IFN-γ rendered them bactericidal (Desai et al., 1989). The activation with interferon gamma -IFN-γ also abrogated the downregulation of Fc receptor expression and HLA-DR antigen expression normally encountered in *M. leprae* infected lepromatous macrophage. It was concluded from studies done on mice that infection with *M. leprae* appeared to partially restrict macrophage by an early
induction of prostaglandins. Biopsies from lepromatous leprosy patients also demonstrated high production of PGE2. It was assumed that similar suppression of IFN-γ activity could exist in lepromatous macrophages which can make them refractory to macrophage activation signals (Sibley and Krahenbuhl, 1987b). However, restoration of various functions such as, Fc receptor expression and HLA-DR antigen expression in lepromatous macrophages by IFN-γ activation did not lead to restoration of in vitro unresponsiveness to M. leprae in lepromatous leprosy patients (Desai et al., 1989). This inference was suggestive of blockade at events such as antigen degradation, processing or presentation.

Few reports support the idea that anergy to M. leprae in lepromatous patients is induced by defective antigen presentation. Macrophages from the lepromatous patients were shown to inhibit the proliferation of HLA-D matched M. leprae responsive lymphocytes. Further, it was observed that lymphocytes of lepromatous patients could respond to M. leprae in presence of macrophages from tuberculoid patients (Hirshberg, 1978; Nath and Singh, 1980). However, a conflicting conclusion was drawn from other experiments (Stoner et al., 1982) which demonstrated no suppression when unresponsive PBMCs isolated from patients with lepromatous leprosy were co-cultured with responsive PBMCs from HLA-D identical healthy siblings in presence of M. leprae. No suppression of lymphoproliferation to M. leprae was evident even when lepromatous PBMCs were in nine fold excess to responsive PBMCs. It was concluded that the sensitized T cell and not the antigen presenting cell is defective element in the lepromatous PBMCs for, good response to M. leprae was observed when adherent cells from lepromatous leprosy patients were co-cultured with T cells from HLA identical responder siblings. This was also confirmed by another report indicating
augmentation of the response to *M. leprae* in PBMCs from lepromatous leprosy patients by functional recovery of CD4+ T cells (Mohagheghpour et al., 1987). Although there have been some reports suggesting release of suppressive factors from lepromatous macrophages which are presumed to interfere with macrophage-lymphocyte interaction, thereby leading to non-responsiveness to *M. leprae*. These suppressive factors released by lepromatous macrophages were shown to suppress the *M. leprae* induced lymphoproliferation of tuberculoid and normal PBMCs to a significant extent (Sathish et al., 1983; Salgame et al., 1983). However, it is believed that the release of such factors is influenced by T cells and it may not be a primary event in series of reactions leading to anergy to *M. leprae*. It was concluded that circulating non T mononuclear cells from non-responder lepromatous leprosy patients could effectively present *M. leprae* antigens to autologous T cells.

Attempts to attribute nonoptimal functions rendered by macrophages / monocytes in lepromatous leprosy patients as plausible mechanisms leading to anergy have so far been inconclusive. It is possible that the aberrations found in lepromatous macrophages are secondary events resulting from *M. leprae* infection which contribute in maintaining anergy rather than inducing anergy. Another line of thought favours the idea that the basic incompetence originates at T cell level.

T Cell Competence

The *in vivo* T cell reactivity against *M. leprae* was found to vary in patients across the leprosy spectrum as demonstrated by histological studies. Differences in microanatomical localisation of helper/inducer (CD4) and suppressor/cytotoxic
(CD8) T cell subsets and differences in ratios of these functionally distinct T cell subsets in peripheral blood and lesions of leprosy patients across the spectrum were again suggestive of dynamic state of T cell reactivity during the course of *M. leprae* infection (van Voorhis et al., 1982; Modlin et al., 1983; Narayanan et al., 1983).

Recently a classic experiment carried out in mice (Gelber et al., 1992) clearly reinforced that an immune response against *M. leprae* is primarily mediated by T cells. Scid mice, known for its inability to form functional B and T cells, when infected with *M. leprae*, developed a significantly more profound foot pad infection than Balb/c mice. Transfer of T cells from *M. leprae* immunized Balb/c mouse resulted in a significant reduction in the number of *M. leprae* found in the foot pad of Scid mouse.

**Immunological Tolerance / Clonal Deletion**

In lepromatous leprosy patients, leukocytes respond to other mycobacterial antigens and also perform efficiently in mixed lymphocyte reaction. However, their failure to respond specifically to *M. leprae* was attributed to the absence of *M. leprae* reactive cells in lepromatous leprosy patient’s T cell repertoire (Godal et al., 1971). It was also suggested that lymphocytes from patients with lepromatous leprosy can respond to *M. leprae* if sensitized lymphocytes are provided in culture. These sensitized lymphocytes were assumed to provide recruiting signals to non-responsive PBMCs from lepromatous leprosy patients (Stoner et al., 1982). However, the existence of immunological tolerance or clonal deletion in lepromatous leprosy patients could not be confirmed by others. These
studies demonstrated that there is no generalised lack of *M. lepraeh sensitized T cells in circulation in patients with lepromatous leprosy, since in vitro responsiveness to *M. lepraeh in lepromatous leprosy patients was restored by co-culturing adherent cells from tuberculoid leprosy patients with T cells of HLA-D compatible lepromatous leprosy patients (Nath et al., 1984a). The T cell unresponsiveness to *M. lepraeh in lepromatous leprosy patients could also be abolished by adding T cell conditioned medium to lymphocyte culture (Haregewoin et al., 1983). These studies clearly indicated that *M. lepraeh sensitized cells are present in circulation of patients with lepromatous leprosy. However, their ability to respond to *M. lepraeh remains suppressed.

**Suppressor T Cells**

Few groups have shown presence of lepromin induced suppressor cells in circulation of lepromatous leprosy patients (Mehra et al., 1979; Nelson et al., 1987). The lepromin induced suppression was found to be mediated by both adherent as well as non-adherent cells from patients with lepromatous leprosy (Mehra et al., 1980). Depletion of non-adherent T cells with TH2+ phenotype resulted in marked enhancement in lymphoproliferation to *M. lepraeh (Mehra et al., 1982). Moreover, the suppressor cell activity disappeared as indicated by an increase in average response to *M. lepraeh when lepromatous leprosy patients were immunized with *M. lepraeh and BCG (Rada et al., 1987). However, contradictory observations have been reported by others. In one such study, *M. lepraeh induced suppression of response to mitogen was found to be a nonspecific phenomenon as it was observed in all patients irrespective of their clinical status (Bjune, 1979). Amount of *M. lepraeh induced inhibition of response to mitogen and number of
patients showing inhibition were found to be, higher in tuberculoid group than in lepromatous leprosy patients or healthy contacts (Nath et al., 1980b). Interestingly, in HLA-D matched co-culture experiments, the lymphoproliferative response was found to be actually above the response of the normal PBMCs. Although, lepromatous PBMC were in 9 fold excess to normal PBMCs. This clearly suggested the lack of any suppressive activity in lepromatous lymphocytes (Stoner et al., 1982). It was also observed that healthy subjects exposed to leprosy for longer period of time showed stronger suppression of the response to \textit{M. leprae} antigens. This led to the conclusion that suppressor cell activity might reflect the maturation of a well regulated and protective immune response required to resist the infection (Stoner et al., 1981). The credence was given to this view by another group indicating that \textit{M. leprae} primed PBMCs from lepromatous leprosy weakly suppressed the proliferation of autologous cells to suboptimal doses of mitogen in comparison with PBMCs from tuberculoid leprosy patients and normal controls. Lepromatous leprosy patients were also shown to have less number of suppressor inducer cells in their circulation than tuberculoid leprosy and erythema nodosum leprosum patients and normal controls (Sasiain, et al., 1989). Lack of normal suppressor activity in patients with lepromatous leprosy was implicated as the reason behind abnormally high immunoglobulin secreting response by their B cells (Bullock et al., 1982). In the recent past, countrasuppressor cell activity (TcS) was detected in patients with lepromatous leprosy (Gonzalez Amaro et al., 1988). When autologous countrasuppressor cells with CD8+ VV+ phenotype were added to mononuclear cells, a significant increase in proliferation to lepromin was observed in patients with lepromatous leprosy. It was paradoxical that despite the presence of countrasuppressor activity, lepromatous leprosy patients’ PBMCs still demonstrated \textit{in vivo} anergy to
It is apparent that the studies on suppressor cell activity in patients with leprosy are very divergent in their views.

**Cytotoxic T Cells**

It has been shown that mycobacteria are very potent inducers of antigen specific CD4+ and CD8+ cytotoxic T cells as well as antigen non-specific killer cells that lyse human monocytes (Kaleab et al., 1990b). Since *M. leprae* inhabit cells of the monocyte/macrophage series, the antigen specific lysis of such cells may be of importance for the elimination of the bacilli. In *in vitro* studies, *M. leprae* induced cytotoxic cells were shown to lyse monocytes isolated from *M. leprae* responsive healthy leprosy contacts and leprosy patients. Most of the lepromatous leprosy patients showed poor responses both with regard to proliferation as well as to induction of cytotoxicity (Kaleab et al., 1990a). Lack of the cytotoxic activity in PBMCs of lepromatous leprosy patients was cited as the probable reason behind the persistence of bacilli in these individuals. However, studies about the role played by cytotoxic T cells in pathogenesis of leprosy are still in their infancy.

**T Cell Derived Effector Molecules**

Determination of efficacy of cytokine mediated effector functions gives a valuable insight into functional behaviour of immune cells. Therefore this area was also not left unattended by researchers in their bid to identify the causative mechanisms leading to pathogenesis in leprosy. There exists a dichotomy on the basis of function and pattern of cytokine secretion in both CD4+ and CD8+ populations. The type 1 lymphokine release of IFNγ, IL2 and lymphotoxin is
mediated by TH1 cells and the type 2 lymphokine release of IL4, IL5, is mediated by TH2 cells. In patients with leprosy, resistance was found to be associated with type 1 cytokine secretion pattern (Salgame et al., 1991). Lesions of the tuberculoid form of the disease reportedly had higher number of transcripts coding for IL2 and IFNγ whereas transcripts for IL4, IL5, IL10, the TH2 like cytokines predominated in the patients with multibacillary form (Yamamura et al., 1991). The T suppressor clones derived from non responder lepromatous leprosy patients were also shown to release predominantly IL4 (Bloom et al., 1992). Further reversal reactions were found to be associated with type 1 cytokine pattern and erythema nodosum leprosum (ENL) with type 2 cytokine pattern (Yamamura et al., 1992). However, in vitro studies have shown considerable degree of heterogeneity of immune response to \( M. leprae \) even in patients belonging to the same clinical category as suggested by conflicting data on cytokine secretion in leprosy patients. The report demonstrating defective production of IL1 by adherent mononuclear cells from patients with lepromatous leprosy (Watson et al., 1984; Horwitz et al., 1984) could not be reproduced in other laboratories (Mohagheghpour et al., 1985). Similarly, attempts to restore in vitro unresponsiveness to \( M. leprae \) by adding exogeneous IL2 led to contradictory results (Haregewoin et al., 1983; Rodriguez et al., 1987; Mohagheghpour et al., 1987). Lymphocytes from lepromatous leprosy patients were shown to be deficient in IFNγ production. It was concluded that IL2 deficiency resulted in subsequent lack of expansion of specifically sensitized T cells which supposedly produce IFNγ and insufficient amount of IFN led to macrophage inactivation, thereby facilitating bacillar growth (Nogueira et al., 1983). However reversal of in vitro anergy to \( M. leprae \) could be achieved only by some laboratories (Nath et al., 1984a; Rodriguez et al., 1987). Others could not repeat this finding (Mohagheghpour et al., 1985; Ottenhoff et al., 1990).
In one report, the failure to restore the \textit{in vitro} responsiveness to \textit{M. leprae} was attributed to inability of lepromatous leprosy patients' PBMCs to acquire functional IL2 receptors (Mohagheghpour \textit{et al.}, 1985). Another report ruled out that IL2 deficiency \textit{per-se} was the causative factor leading to anergy in lepromatous leprosy patients, as addition of an exogeneous IL2 could only expand already sensitized cells as shown by augmentation of \textit{in vitro} responses in hyporesponder lepromatous leprosy patients. Addition of IL2 did not lead to \textit{de-novo} sensitization on its own as shown by failure of exogeneous addition of IL2 to restore \textit{in vitro} proliferative responses of nonresponder lepromatous leprosy patients (Ottenhoff \textit{et al.}, 1984b; Kaplan \textit{et al.}, 1985a,b). Reports on secretion of TNF\(\alpha\), another macrophage activating factor in leprosy patients were again contradictory. High production of TNF\(\alpha\) was reported in patients with tuberculoid leprosy (Silva \textit{et al.}, 1989; Barnes \textit{et al.}, 1992a). However, others observed elevated levels of TNF\(\alpha\) in lepromatous leprosy patients and low levels in tuberculoid form of leprosy (Parida \textit{et al.}, 1992).

\textbf{Mycobacterium Derived Factors}

\(H-2^b\) mice, when immunized with Chicken lysozyme molecule (HEL) did not show T cell proliferation against this molecule. However a vigorous T Cell response was mounted by cells from immunized mice on stimulation of their cells with a peptide derived from HEL (Yowell \textit{et al.}, 1979). It was concluded that a restricted determinant present on the molecule can drive some cells to suppress the response to the majority of the molecule. It was assumed that similar situation could exist in leprosy. There might exist some antigens in \textit{M. leprae} which elicit suppression of proliferative response to whole bacilli. This presumption was
supported by a report implicating *M. lepra* antigens for their role in maintaining unresponsiveness in patients with lepromatous leprosy. CD4⁺ T cells from nonresponder lepromatous leprosy patients, on preincubation with *M. lepra* were shown to proliferate to a lesser extent as compared with cells which were precultured in medium alone (Mohagheghpour et al., 1987). Further, when challenged with purified *M. lepra* proteins, a remarkable increase in efficacy of recombinant IL-2, to restore the responsiveness in PBMCs from patients with lepromatous leprosy was seen (Ottenhoff et al., 1989). The stimulating effect observed at low doses and reduced proliferation at higher doses of *M. lepra* in a borderline tuberculoid leprosy patient, again suggested existence of both helper and suppressor epitopes in *M. lepra* antigens (Kaplan et al., 1987).

It was hypothesised that *M. lepra* antigens inhibited the immunological recovery of T Cells from lepromatous leprosy patients either by remaining bound to antigen reactive cells or by modulating the T cell receptor, thus making the cells temporarily tolerant. Another possibility put forward to explain the antigen induced anergy was that *M. lepra* induced the suppressor cells which prevented the proliferation of antigen reactive CD⁺ helper cells (Mohagheghpour et al., 1987).

**Suppression Inducing Determinants**

The lipopolysaccaride-lipoarabinomannan (LAM) - a mycobacterial cell wall polysaccharide was shown to inhibit mitogen/antigen induced responses in patients with borderline tuberculoid and lepromatous leprosy as well as controls (Molloy et al., 1990). This suppression of *in vitro* proliferation by LAM was
found to be mediated by nonspecific soluble factors such as lymphokines as indicated by LAM induced inhibition of PHA/PMA induced accumulation of transcripts for IL-2, IL-3, GMCSF and IL-2 Rα in human Jurkat cell line (Chujor 
et al., 1992). The suppressive activity of LAM was attributed to the nonproteinaceous heat stable, water soluble lipopolysaccharide content of LAM (Molloy 
et al., 1990).

PGL-1, a M. leprae specific component present abundantly in the isolated bacillus, sera, urine and skin biopsies of patients with leprosy has also been shown to induce suppression of the immune response (Hunter et al., 1981; Young et al., 1985; Cho et al, 1986). Normal monocytes, when treated with PGL-1 showed decreased level of superoxide anions (Vachula et al., 1989). PGL-1 also induced suppression of mitogenic responses of PBMCs from patients with lepromatous leprosy. It was concluded that PGL-1 is at least one of the major suppression inducing determinants of M. leprae as monoclonal antibodies specific for terminal disaccharide moiety of M. leprae glycolipid were able to completely abolish the PGL-1 induced suppression (Mehra et al., 1984).

**Immunogenic Determinants**

Cell walls of M. leprae, consisting of complex arrangements of carbohydrates, lipids, peptidoglycans and protein molecules have been shown to contribute significantly to cell mediated immunity. Lymphocytes from tuberculoid leprosy patients were shown to respond as well to purified cell walls as they did to the intact bacillus. Highly purified cell wall core of M. leprae was shown to elicit delayed hypersensitivity (DTH) reactions in guinea pig. Tuberculoid leprosy
patients were also found to react strongly with the cell wall peptidoglycan (PPC) free of mycolates and arabinogalactans (Kaplan et al., 1988). CD4+ cell lines derived from tuberculoid leprosy patient lesions responded equally well to whole \textit{M. leprae} and cell wall core of \textit{M. leprae} whereas CD4+ T cell lines derived from lepromatous leprosy lesions failed to respond to both whole \textit{M. leprae} as well as to the cell wall core (Mehra et al., 1989). It was concluded that the cell wall of \textit{M. leprae} contains major antigens involved in cell mediated immunity and delayed hypersensitivity reactions.

The major handicap of not being able to cultivate \textit{M. leprae} in vitro has now been circumvented by cloning of \textit{M. leprae} genome in a recombinant DNA expression library (Young et al., 1985). Genes for the most immunogenic protein antigens of the leprosy bacillus -65 kDa, 36 kDa, 28 kDa, 18 kDa and 12 kDa have been identified. T cell lines from tuberculoid leprosy patients or contacts of leprosy patients, when tested for their reactivity to \textit{M. leprae} sonic extract separated on gradient SDS-PAGE, frequently recognised the proteins of M.wt. 7 kDa, 16 kDa, 28 kDa, 18 kDa and 65 kDa. These proteins were found to be components of the most highly purified cell wall proteins (Mehra et al., 1989).

Mycobacterial heat shock proteins such as Hsp70, Hsp65, Hsp10 accumulated inside the macrophages in response to oxidative and other environmental stress conditions were also shown to elicit significant T cell response in patients with tuberculoid leprosy and normal contacts (Munk et al., 1989; McKenzie et al., 1991; Mehra et al., 1992), whereas lepromatous leprosy patients were found to react negligibly to these proteins (Mehra et al., 1992). \textit{M. leprae} specific helper T cell clones established from tuberculoid leprosy patient
also recognised an antigenic determinant expressed on purified \textit{M. leprae} 36 kDa protein containing high content of proline rich repeats (Thole \textit{et al.}, 1990). However, response to 36 kDa protein was inhibited by \textit{M. leprae} unreactive T cell line established from a non responsive borderline lepromatous patient (Ottenhoff \textit{et al.}, 1986a). It has been shown lepromatous leprosy patients who failed to respond to the whole soluble extract of \textit{M. leprae}, showed lymphoproliferation following stimulation with fractionated antigens. \textit{M. leprae} unresponsiveness in lepromatous leprosy patients could be successfully abolished by \textit{in vitro} rechallenge with \textit{M. leprae} components separated by either one dimensional or two dimensional SDS-PAGE (Ottenhoff \textit{et al.}, 1989, Gulle \textit{et al.}, 1992). These observations were again suggestive of the fact that suppressor entities exist in \textit{M. leprae}. However, another study failed to observe any enhancement of response by fractionated \textit{M. leprae} (Samperio \textit{et al.}, 1989). In another study, a wide variety of profiles with no reproducible patterns of responses to fractionated antigens was observed within either group. Almost every fraction was found to stimulate proliferation with at least one donor (Lee \textit{et al.}, 1989). Responses to the 22-19 kDa and 17-15 kDa were found more frequent in tuberculoid than in lepromatous families (Samperio \textit{et al.}, 1989). Another study also showed predominant responses of T cells in majority of tuberculoid patients to \textit{M. leprae} antigens in the lower molecular weight range 10-25 kDa (Ottenhoff \textit{et al.}, 1989). Borderline tuberculoid patients were found to respond to 36 kDa and high molecular weight antigens (100 kDa or more) of \textit{M. leprae} (Filley \textit{et al.}, 1989). However, undetectable or only weak responses to any fraction of two dimensional polyacrylamide gel electrophoresis (2D-PAGE) separated \textit{M. leprae} antigens were observed with T cells from untreated borderline tuberculoid patients (Gulle \textit{et al.}, 1992). Antigens in the M.wt. range of $> 150$ kDa were found to be uniquely seen
by lepromatous patients. Lepromatous patients were also shown to respond to low molecular weight antigens, however, they failed to respond to recombinant 18 kDa antigen (Dockrell et al., 1989). Long term treated leprosy contacts were shown to respond to the recombinant 18 kDa antigen, more strongly than the tuberculoid leprosy patients (Dockrell et al., 1989). The contacts were shown to respond to several antigens in the 18-35 kDa range (Filley et al., 1989). Peripheral blood lymphocytes of healthy contacts of patients in a leprosy endemic country, in another study, were shown to mount a more prominent response against M. leprae antigens in the higher molecular weight ranges of 22-26 kDa and 45-66 kDa. Moreover, a highly significant correlation was observed between the ability of fractions to induce proliferation and interferon production (Converse et al., 1988). However, in another report, responses to the 65 kDa were found to prevail in both lepromatous cases and their familial contacts than tuberculoid cases (Samperio et al., 1989).

C. GENETIC PREDISPOSITION

A number of strides made to understand the intricacies in immunological mechanisms involved in pathogenesis of leprosy has helped us immensely in gaining better insight into different functions rendered by immune cells under normal and pathological conditions. However, it still remains an enigma why the vast majority of people exposed to M. leprae do not develop the disease; and those who do and become lepromatous, respond to other mycobacterial antigens but not to M. leprae. Assuming that the inherent evasive ability of M. leprae helps it in giving a slip to the defence mechanisms in patients with lepromatous leprosy, the inability of M. leprae to protect itself in tuberculoid and normal macrophage still
remains unexplained. Further, number of queries such as, why tuberculoid patients need to spend extra immune energy to kill the bacilli; what are the factors which predispose patients to become lepromatous or tuberculoid, probably cannot be answered by observations assimilated so far. It is inconclusive from evidences whether available observations discriminating immune responses of leprosy patients and normal healthy individuals or lepromatous and tuberculoid individuals, reflect the fundamental defect or just the perturbations in immune system resulting from some basic aberrations. Moreover, more than once these observations have run into conflicts, suggesting that there exists a heterogeneity of immune response even among patients belonging to same clinical group.

Genetic Variations and Impaired Immune Response

The variation found in immune response of patients across the leprosy spectrum could not be attributed to \textit{M. leprae} derived factors since no known form of genetic variability has been found to exist in \textit{M. leprae} (Shepard and McRae, 1971). Instead, there are several lines of epidemiological evidences which give credence to the view that it is the genetic make up of an individual in the leprosy spectrum which may determine the relative position of an individual in the spectrum. The observations of an extraordinary high concordance for leprosy in monozygotic twins, familial aggregation and vertical transmission of leprosy in multiple generations, favour the role of genetic factors in predisposing individuals to leprosy (Ali and Ramanujan, 1966; Chakravarti and Vogel, 1973). Also, segregation of a recessive major gene for lepromatous and nonlepromatous leprosy suggested by some (Smith, 1979; Haile \textit{et al.}, 1985) and not supported by others (Shields \textit{et al.}, 1987) has been proposed. However, the mechanism(s) of action of
such genetic determinants and the degree to which they control the pattern of disease in humans are not yet completely understood.

**HLA Immune Response Genes**

The fact that HLA encoded molecules mediate the specific interaction between T cells and macrophages in an immune response, provided an impetus to researchers to explore HLA encoded genetic control of the immune response in leprosy patients. Associations between HLA antigens and certain other infectious diseases in man have already been documented (McDevitt and Bodmer, 1974). Initial HLA haplotype segregation data from different studies demonstrated excess of shared haplotypes among both tuberculoid leprosy and borderline lepromatous, lepromatous leprosy affected sibs.

Recently a significant increase in BW-60-HLA class I antigens has been reported in lepromatous leprosy patients as compared with borderline lepromatous patients (Rani et al., 1992). However, a number of attempts made in the past, to relate HLA class I antigens - HLA-A, HLA-B, HLA-C with different leprosy types, failed to find significant associations between the HLA antigens and leprosy (Kreisler et al., 1974; Dasgupta et al., 1975). Instead, studies carried out to analyse associations between HLA class II molecules and leprosy types have been more consistent in providing a convincing data. A preferential segregation of HLA Class II-DR2 in tuberculoid leprosy affected sibs has been demonstrated in Indian multiple case families. A significant association found in families of healthy parents with sibs affected with tuberculoid leprosy was suggestive of recessive mode of action of this DR-2 associated genetic factor (Fine et al., 1979). DR-2,
DQ 1 and DR-2, MT-1 have been reported to be strongly associated with tuberculoid leprosy in patients from Thailand and Japan (Miyanaga et al., 1981; Schauf et al., 1985). However in Japanese patients with tuberculoid leprosy, increase in frequency of DR antigens was attributed to a linkage disequilibrium between MT and DR2 antigens. Tuberculoid leprosy in these patients was found to be more strongly associated with MT-1 than with DR-2. It was concluded from other studies also, that DR-2 might not be the susceptibility gene itself (deVries et al., 1980).

Multiple skin testing with mycobacterial antigen preparations in healthy individuals demonstrated absence of HLA DR-3 in haplotype of individuals who did not show delayed hypersensitivity reactions (DTH) to tuberculin. It was presumed that HLA-DR3 might confer some degree of DTH high responsiveness (van Eden et al., 1983a). Preferential inheritance of HLA DR3 has also been reported in tuberculoid leprosy patients from various ethnic groups of Venezuela (van Eden and deVries, 1984), whereas lepromatous leprosy patients from the same place showed an increase of the HLA LB-E12 allele which is similar to or identical with MB1, DC1 and MT1 (Ottenhoff et al., 1984a).

T cell proliferative responses to M. leprae could be inhibited by pretreatment of responding T cell population by monoclonal antibodies against HLA class II antigens (Haregewoin et al., 1983). Further inhibition studies using well defined HLA class II specific monoclonal antibodies showed that the majority of the restriction elements for M. leprae reside on DR and not on DP or DQ molecules (Ottenhoff et al., 1986b). These studies clearly indicated that there exists differences in the capacity of distinct HLA-DR determinants to serve as
restricting elements in cellular interactions occurring in reactions to mycobacterial antigens. However, expression of HLA encoded susceptibility conferring gene could not be detected by lymphocyte transformation tests which showed similar pattern of *in vitro* reactivity to *M. leprae* in different groups of individuals who are either fully HLA identical or non HLA identical with their sibs affected with tuberculoid leprosy (van Eden *et al.*, 1983b).

It was found that the segregation of HLA haptotypes shared between leprosy patients in a given sibship did not occur less frequently among the healthy siblings of that sibship as seen in Indian, Venezuela and Chinese populations (van Eden and deVries, 1984). Complex segregation analysis, performed on 27 multigenerational pedigrees from Desirade implicated the presence of a recessive or codominant major gene controlling susceptibility to leprosy per se and nonlepromatous leprosy, respectively (Abel and Demenais, 1988). However, no significant linkage between these genes and five markers-HLA, ABO, Rhesus, Gm, Km was detected (Abel *et al.*, 1989).

It can be concluded from these studies that HLA linked genes may control in part the type of leprosy that may develop upon infection but they do not control susceptibility to leprosy per se. Thus, the associations between leprosy and HLA antigens were found not to be strong and a considerable amount of population heterogeneity was evident in these reports. These inferences suggested that the primary susceptibility gene for leprosy may be at a locus closely linked with HLA, rather than HLA itself.
Non HLA Genes

A considerable amount of population heterogeneity observed in HLA studies compelled researchers to presume that the primary susceptibility gene for leprosy may lie outside the HLA gene complex. Taking a cue from observations which are suggestive of role of single autosomal gene Bcg/Lsh/Ity in *M. lepremurium* infection in mice, attempts have been made to identify the syntenic gene with similar functions on human chromosome number 2 (Schurr *et al.*, 1990). In another study, no evidence was found at the population level for an association between leprosy susceptibility and polymorphism in human γ crystalline genes, a syntenic group with Isocitrate dehydrogenase-1 in both mouse and man. (Jazwinska and Serjeantson, 1988).

Recently, Nramp (natural resistance-associated macrophage protein) has been identified as human homologue of murine Bcg gene (Vidal *et al.*, 1993). Despite the cloning of this gene, the exact mechanism of genetic resistance to *M. leprae* in humans remains unidentified.

T Cell Receptor (TCR) Genes

T cell receptor molecules play a pivotal role in cell mediated immune response against various pathogens. Genetic susceptibility due to T cell receptor could arise at two levels (Fig.1), either within the genomic DNA encoding the structural and regulatory elements of the receptor or from T cell receptors recombined and selected after the influence of somatic events associated with rearrangement of gene segments, N region addition, structural element editing (Moss *et al.*, 1992).
T CELL RECEPTOR ENCODED SUSCEPTIBILITY

\[ \alpha \beta \text{T CELLS} \quad \gamma \delta \text{T CELLS} \]

\[ \alpha, \beta \dddot{\text{AAA}} \quad \gamma, \delta \dddot{\text{AAA}} \]

\[ \alpha, \beta \dddot{\text{S, N}} \]

\[ \beta \dddot{\gamma} \]

\[ \text{CD}3^+ \text{CD}4/8^+ \]

\[ \text{CD}3^+ \text{CD}4^+\text{CD}8^-\text{?}^+ \]

\[ \text{CD}3; \bigtriangleup \text{\(\alpha\beta\) TCR}; \bigcirc \text{CD}4/\text{CD}8; \blacksquare \text{Ag}; \bigtriangleup \text{\(\gamma\delta\) TCR} \]

GENETIC SUSCEPTIBILITY
(GERMLINE CONFIGURATION)

GERMLINE POLYMORPHISM
COMBINATORIAL DIVERSITY

ALLELIC VARIATION IN
REARRANGED NON FUNCTIONAL
ST/REG ELEMENTS OF TCR
FORMS, ABNORMAL EXPRESSION

RFLP (DNA POLYMORPHISM)
ALTED IMMUNE RESPONSE
ALTED TCR

DIFFERENT TCR REARRANGEMENTS

Fig. 1
T cells use either of the two different receptors to recognise antigens, T cell receptor alpha-beta (TCR $\alpha\beta$) or T cell receptor gamma delta (TCR $\gamma\delta$). The major population of mature T cells in circulation bear TCR $\alpha\beta$, a clonally variable, disulfide linked heterodimer consisting of $\alpha$ and $\beta$ subunits (Allison and Lanier, 1987). This major population of mature $\alpha\beta$ T cells, recognise antigens in the context of self major histocompatibility (MHC) molecules (Meuer et al., 1983). The role played by $\alpha\beta$ T cells in antigen / MHC recognition is well understood. However, the knowledge about minor T cell population, comprising 1% to 10% of mature CD3$^+$ T cells bearing a heterodimer of gamma ($\gamma$) and delta ($\delta$) TCR chains, with respect to their antigen specificity, genetic restriction, functional activities and physiological roles, is scanty (Borst et al., 1987; Brenner et al., 1986).

The accumulating evidences indicate that $\gamma\delta$ T cells and $\alpha\beta$ T cells recognise antigens differently and function distinctly in the primary immune response. The data available on the role of MHC molecules in antigen presentation to $\gamma\delta$ T cells is conflicting. Evidence suggesting no influence (Holoshitz et al., 1989; Janis et al., 1989; O'brien et al., 1989) or an equivocal influence (Haregewoin et al., 1989; Kozbor et al., 1989; Modlin et al., 1989) of classical MHC molecules has been reported. Earlier reports demonstrated lysis of a variety of tumor target cells by human $\gamma\delta$ T cell lines regardless of their MHC antigen expression. A variety of monoclonal antibodies directed against MHC Class I or Class II determinants failed to inhibit lysis of target cells by $\gamma\delta$ T cell lines (Brenner et al., 1987). However another line of thought favours that $\gamma\delta$ T cells are capable of self-nonself MHC discrimination. To support this idea, anti class I monoclonal antibodies were shown to inhibit specific lysis of allogenic cells by $\gamma\delta$
cells (Ciccone et al., 1988). In another report, specific proliferative responses of \( \gamma \delta \) T cells to tetanus toxoid and autologous antigen presenting cells were found to be restricted by HLA DR4 related element (Kozbor et al., 1989). Recently, it has been shown that \( \gamma \delta \) T cells recognise a particular HLA DQ \( \alpha/\beta \) heterodimer with a specificity closely resembling that of \( \alpha/\beta \) T cells (Bosnes et al., 1990). It is concluded that \( \gamma \delta \) T cells can undergo MHC influenced selection during differentiation like \( \alpha/\beta \) T cells.

There have been number of reports which are suggestive of propensity of \( \gamma \delta \) T cells to preferentially recognise mycobacterial antigens. *Mycobacterium tuberculosis* has been shown to induce in vivo activation of \( \gamma \delta \) T cells followed by IL-2 receptor expression. Also, a greater proliferative response of \( \gamma \delta \) T cells to IL-2 was observed in mice primed with mycobacterial antigens (Janis et al., 1989). An increase in the number of \( \gamma \delta \) T cells was reported in athymic nude mice treated with complete Freund's adjuvant-a preparation containing mycobacterial components (Yoshikai et al., 1990). \( \gamma \delta \) T cell lines reactive with mycobacterial antigens and tuberculin PPD have been generated from leprosy skin lesions (Modlin et al., 1989), synovial fluid of a rheumatoid arthritis patient (Holoshitz et al., 1989) and a PPD immunized healthy individual (Haregewoin et al., 1989). \( \gamma \delta \) T cells present in the cord blood of new born infants have also been shown to proliferate in vitro upon stimulation with either heat killed mycobacterial organisms or their lipid fraction in the presence of adherent cells. These components were found less stimulatory or nonstimulatory in tuberculin skin test positive individuals (Tsuyuguchi et al., 1991). In another report, monocytes treated with live *Mycobacterium tuberculosis* were found to be very effective inducers of \( \gamma \delta \) T cell expansion, whereas heat killed *Mycobacterium tuberculosis*
expanded αβ T cells. It was concluded that γδ T cells may therefore exert an important role in the initial immune response to mononuclear phagocytes infected with living intracellular bacteria (Havlir et al., 1991). Thus γδ T cells may participate in immune surveillance as a first line of defense against the invasion of mycobacterial antigens. An intriguing hypothesis is that γδ T cells have been evolutionally selected to respond to certain mycobacterial antigens, thus enabling this population to respond quickly before the population of antigen specific αβ T cells begins to expand (Tsuyuguchi et al., 1991).

In leprosy, γδ T cells were found to accumulate in particular granulomatous reactions (Modlin et al., 1989). Expansion of γδ T cells by M. tuberculosis was found lesser in lepromatous than in tuberculoid leprosy patients, despite equivalent αβ T cell responses, though the number of γδ T cells were found to be similar in skin lesions of lepromatous and tuberculoid leprosy (Barnes et al., 1992b). It was presumed that γδ T cells may contribute to resistance against mycobacterial infection through effector function such as secretion of macrophage activating cytokines such as IFNγ, GMCSF, IL-3 and TNFα and negligible amounts of IL-4 and IL-5 (Modlin et al., 1989). Since the pattern of cytokine secretion was similar to that of αβ T cells, it was concluded that a specific role for γδ T cells in mediating immune response to mycobacterial infection probably did not reside in unique effector function but depended on recognition of antigens, epitopes or restriction elements distinct from those recognised by αβ T cells (Modlin et al., 1989).

Mycobacterial antigens have been shown to stimulate γδ T cells in a receptor dependent fashion. This inference was drawn from the observation which demonstrated anti CD3 induced inhibition of spontaneous production of
interleukin-2 by γδ hybridomas (O'Brien et al., 1989). Further, specific antigens were shown to be recognised by a limited group of γδ receptors, as interleukin-2 was produced spontaneously only by those γδ hybridomas which expressed Vδ6 gene product unlike Vδ1+ hybridomas which did not produce interleukin-2 spontaneously. Specificity of antigen recognition by TCR γδ was further highlighted by an observation implicating a requirement of specific gamma chain together with a limited set of delta chains for reactivity of γδ hybridomas to PPD nonreactive population (Happ et al., 1989). γδ T cell lines established from PPD immunized individuals were found to respond vigorously to PPD and recombinant heat shock protein 65 KD only in the presence of autologous APC (Haregewoin et al., 1989). γδ T cells failed to respond to mycobacterial antigens when T cell depleted autologous cells were used as APC. However, the responsiveness of γδ T cells to *M. tuberculosis* was fully restored either by reconstituting cultures with purified CD4 T cells or by adding IL2, a CD4 cell derived helper factor (Pechhold et al., 1994). The nature of ligands recognised by γδ T cells and CD4+ T cell lines expanded by *M. tuberculosis* were found to be quite distinct. γδ T cells have been shown to respond selectively to the whole mycobacteria, unlike CD4+ T cells which were found to recognise both secreted protein antigens as well as whole mycobacteria (Boom et al., 1992). Recently it has been demonstrated that unlike αβ T cells, γδ T cells respond to protease resistant, lectin binding mycobacterial ligands contained in < 10 kDa fraction in the presence of APC expressing class II MHC antigens especially HLADR (Pfeffer et al., 1990; 1992).

**Genomic organization of TCR γδ**

Like the TCRα and β chain, the germline DNA for gamma and delta loci is split into distinct variable (V), Diversity D (of delta chain) and joining (J) gene
segments. These segments are rearranged during T cell development to form the portion of receptor chains that imparts diversity (Brenner et al., 1987).

Human T cell receptor gamma (TCRγ) locus, located on chromosome 7 at band 7p15 (Lefranc et al., 1985; Murre et al., 1985), is composed of 14 variable, 5 joining gene segments and two constant region genes, Cγ1 and Cγ2. Variable (Vγ) gene segments are divided into four subgroups - VγI, VγII, VγIII and VγIV based on sequence similarity (Lefranc and Rabbitts, 1989). Subgroup I contains 5 functional V gene segments and four nonfunctional pseudogenes, whereas the more downstream subgroups II, III and IV each consists of a single Vγ gene segment designated as Vγ9, Vγ10, Vγ11, respectively. These V subgroups differ in sequence from each other (Triebel et al., 1988).

Three J segments (JP1, JP and J1) are located upstream of Cγ1 and 2 J segments (JP2 and J2), upstream of Cγ2. The Jγ1 cluster includes Jγ1.1, Jγ1.2 and Jγ1.3 and Jγ2 cluster includes Jγ2.1 and Jγ2.3. Jγ1.3 and Jγ2.3 encode identical amino acid sequence. JP segment is located upstream of J1 (Lefranc et al., 1986a). Two additional J segments -JP1 and JP2 have been located in the Cγ1 and Cγ2 loci, respectively (Quertermous et al., 1987). Cγ1 gene is composed of three exons. A cysteine residue required for disulfide linkage to the δ chain is encoded by the second exon. Cγ2 gene contains five exons including three tandem copies each of which is similar in sequence to the second exon of Cγ1 but lacks a cysteine residue. Distinct genetic Cγ loci encoding structurally distinct TCR γ polypeptides account for three protein forms of the γδ T cell receptor (Brenner et al., 1986; 1987; Borst et al., 1988; Hochstenbach et al., 1988). The variation in structure of human TCR γδ form is unprecedented among TCR as no such
parallel is observed in TCR $\alpha\beta$ (Toyonaga and Mak, 1987). In form I of TCR $\gamma\delta$ - a 40 kDa TCR $\gamma$ protein encoded by $C_\gamma I$ gene is disulfide linked to the TCR $\delta$ protein. In contrast, two nondisulfide linked forms display either a 40 kDa (form 2bc) or 55 kDa TCR $\gamma$ chain (form 2abc) in association with the TCR delta chain (Band et al., 1989). TCR $\gamma$ polypeptides of forms 2 bc and 2abc correlate with the usage of allelic forms of the $C_\gamma 2$ gene segment (Krangel et al., 1987; Lefranc et al., 1986b).

The human $\delta$ locus has also been characterized. The $\delta$ locus is located on chromosome 14, approximately 85 kb 5' of the $C_\alpha$ genes (Boehm et al., 1988). A single $C_\delta$ gene, $3I_\delta$ gene segments $I\delta 1$, $I\delta 2$, $I\delta 3$; three diversity (D) delta gene segments $D\delta 1$, $D\delta 2$, $D\delta 3$ and at least 5 unique $V\delta$ genes have been identified in humans (Hata et al., 1987). Since the TCR $\alpha$ and TCR $\delta$ gene segments have the same transcriptional orientation, it was concluded that same V region pool may be shared by $\alpha$ and $\delta$ genes (Chien et al., 1987). $V\delta 3$ in humans has been found to be located 3' to $C_\delta$ gene in the opposite transcriptional orientation (Korman et al., 1989).

Thus a major difference between $\alpha\beta$ and $\gamma\delta$ T cells is the number of available germline gene segments coding for variable and joining regions of the respective TCR chains. In striking contrast to large number of potentially available $V_\alpha$ and $V_\beta$ elements for $\alpha\beta$ T cell repertoire, only a small number of $V_\gamma$ and $V\delta$ segments are available in the germline (Lefranc and Rabbitts, 1990). However, additional mechanisms such as the insertion of nucleotides at the V-J junctions contribute to the diversity of the $\gamma\delta$ T cell repertoire which is probably in the same order of magnitude as the repertoire of $\alpha\beta$ T cells (Strominger, 1989).
Rearrangement and Expression of TCR γδ Genes:

The γδ genes undergo rearrangement and transcription in the fetal thymus prior to β chain rearrangement during T cell differentiation. V to D rearrangement appear to precede D to J rearrangement in the δ locus while D to J rearrangement precede V to D rearrangement in the β locus (Toyonaga and Mak, 1987). The rearrangement of TCR γ and δ gene segments are mediated by recognition signals such as conserved heptamer and nonamer sequences separated by conserved spacer. A highly conserved heptamer is located proximal to each of the coding V, D and J segments followed by a nonconserved spacer and then a conserved AT rich nonamer. The nonconserved spacers can have length corresponding to either 12 nucleotides as observed in signals 5' to Dδ, Jγ and Jδ segments or 23 nucleotides as observed in signals 3' to the Vγ, Vδ and Dδ segments. The most common rearrangement mechanism for TCR γ and δ genes involves formation of a stem loop structure between recognition signal sequences. The stem is produced by basepairing between the heptamer and nonamer sequences and the loop is comprised of the DNA between segments being joined. Inversion is another rearrangement mechanism in which an inverted segment moves into a position beside a segment in the opposite orientation. This occurs during rearrangement of Vδ3 genes located 3' to Cδ in the opposite transcriptional orientation. The imprecision of the joining process and the addition of short stretches of nucleotides called N regions at the VγJγ and VδDδJδ junctions during joining process results in significant variability at many V-J and V-D-J junctions (Yoshikai, 1991).
Preferential Usage of Specific Segments by TCR Genes in γδ T Cells

About 80% of γδ T cells in peripheral blood were found to express Vγ9. And 83% of these Vγ9 bearing cells coexpressed Vδ2 (Schondelmaier et al., 1993). TCR γδ+ subpopulation was shown to express either a TCRγ1 disulfide linked receptor or a nondisulfide linked TCRγ2 receptor. TCRγ1+ cells were found to express a γ chain encoded by Vγ9 gene rearranged to JP. This γ chain was found to be predominantly associated with a δ chain encoded by Vδ2/D/Jδ1 rearranged gene. An additional infrequent TCR γ1+ subpopulation was shown to express a receptor with γ chain encoded by rearrangement either not involving V9 or involving the joining of V9 to J1 (Krangel et al., 1987). In the TCR γ2+ subpopulation, productive Vγ9/Vγ1 rearrangement was found to be rarely expressed. Instead, various Vγ genes often from subgroup I were found to be employed, thus generating more combinatorial diversity. With respect to δ, a Vδ1 D/Jδ1 chain was found to be expressed predominantly on TCR γ2+ lymphocytes.

Investigations using TCR specific monoclonal antibodies revealed exclusive expression of Vγ9/Vδ2 bearing TCR by mycobacteria responsive γδ T cells. However, the coexpression of Vγ9 and Vδ2 was found not to be genetically determined as Vγ9+/Vδ2+ in human peripheral blood was presumed to be driven by a specific antigen challenge (Parker et al., 1990). Interestingly, only TCR γ1+ cells expressing Vγ9+/Vδ2+ were found to proliferate in response to PPD in PPD reactive individuals (Miyawaki et al., 1990). Further it was shown that response to Mycobacterium tuberculosis in cultures, selectively depleted of Vγ9 bearing cells was shown to be mediated exclusively by αβ. Further, blocking
of the primary response of $\gamma\delta$ T cells using anti $V\gamma9$ monoclonal antibody and preferential outgrowth of $V\gamma9 / V\delta2$ cells in cocultures of $\gamma\delta$ thymocytes containing a larger proportion of $V\gamma1^+$ cells suggested that response to mycobacterial antigens was an exclusive property of $V\gamma9$ bearing cells (Kabelitz et al., 1991). A prior specific antigenic challenge was not found to be prerequisite for the antimycobacterial reactivity of $\gamma\delta$ T cells since $\gamma\delta$ T cells were found to expand in both PPD sensitized / unsensitized individuals as well as foetal and adult PBMC cultures. It was concluded that mycobacterial reactivity might be TCR $\gamma$ or $\delta$ germline gene encoded (Panchamoorthy et al., 1991). Selective engagement with a TCR variable gene segment of $\gamma\delta$ T cells and the use of class II MHC molecules as presenting structures were suggestive of role of mycobacteria as superantigen for human $\gamma\delta$ T cells.

There have been some attempts to analyse genetic susceptibility arising from recombined and selected T cell receptors in leprosy patients. Specific TCR V$\beta$ populations were found to be over represented in lesions from reversal reactions (Wand et al., 1992). In another study, it has been shown that mainly V$\beta5$ gene segment was used by DR-3 restricted clones whereas V$\beta18$ was preferentially employed by DR-2 restricted clones within the panel of $M. leprae$ reactive T cell clones derived from a tuberculoid leprosy patient (van Schooten et al., 1992). These studies implicated role for specific gene segments of rearranged TCR in antimycobacterial reactivity in leprosy patients.

When analysed for TCR diversity of $\gamma\delta$ T cells in leprosy, it revealed that V$\delta1$ and V$\delta2$ bearing cells accounted for the majority of infiltrating $\gamma\delta$ cells with a V$\delta2 / V\delta1$ ratio of 2:1 within the dermal granulomas from lepromin skin tests.
This was in contrast to V\(\delta2 / V\delta1\) ratio of 9:1 in peripheral blood of the same individuals. Unlike \(\gamma\delta\) T cells present in the peripheral blood and dermis, \(\gamma\delta\) T cells infiltrating the epidermis were found to primarily express V\(\delta1\) encoded delta chain (Uyemura et al., 1991). The majority of V\(\delta1\)-J\(\delta1\) and V\(\delta2\)-J\(\delta1\) junctional sequences were found to be identical in lepromin skin tests in contrast to junctional sequences of products obtained from peripheral blood of these patients which exhibited extensive diversity. The greater amino acid sequence diversity at the V-J junction between individuals than between clones within an individual was indicative of role of primary genetic difference in repertoire generations (Uyemura et al., 1991).

Germline polymorphism of T cell Receptor genes might lead to the existence of new allelomorphs. RFLPs arising because of germline variations at the T cell receptor loci can be related to the disease caused by gene defect in at least three different ways. The mutation causing the disease may itself give rise to the RFLP. Alternatively, the RFLP may arise within the locus of the susceptibility gene independent of the mutation that causes the disease. Finally, the RFLP may not lie within the locus of the gene of interest at all, but may lie close enough to the gene so that the two loci rarely are separated by recombination. A TCR chain with unusual conformation, normally nonpermissive, might be encoded by such polymorphic genes which when expressed in context of HLA gene would lead to an impaired immune response.

A possible T cell receptor defect in leprosy patients was searched by examining RFLPs in the TCR \(\alpha\), \(\beta\) and \(\gamma\) genes. These RFLPs were not found to be associated with leprosy subsceptibility (Jazwinska and Serjeantson, 1988).
However, this study was carried out on subjects including mostly lepromatous cases. Therefore, it remained elusive whether there existed any difference at T cell receptor loci within leprosy patients across the whole spectrum. In short, no extensive search has been carried out till date, to explore the potential germline defect at TCR gamma and delta loci in leprosy patients.