INTRODUCTION
A. LEPROSY - THE DISEASE

PREVALENCE AND IMPORTANCE

Leprosy continues to be major physical disabling condition in the world. More than 5.4 million people in tropical and sub-tropical regions of the world are saddled with this suffering. (Noordeen et. al. 1992). Over 82% of the registered cases are accounted in five countries: India, Brazil, Nigeria, Myanmar and Indonesia. India ranks the foremost and the incidence of leprosy is vast, both in number (5 per thousand) and its geographical distribution in over 201 districts (Hind Kusht Nivaran Sangh Report 1992). The areas of high prevalence are found mainly in the South-Eastern and Central regions of India including Tamil Nadu, Andhra Pradesh, Orissa, Bihar, Madhya Pradesh, Uttar Pradesh, Maharashtra and West Bengal. In some endemic areas, the incidence rate for early skin lesions of leprosy are bimodal; the first peak is observed in the age group of 10-14 years and the second in 30-40 years. The disease, in general, is reported to be more frequent in males than in females, with a ratio of about 2:1 (Jod et. al. 1991).

With the advances in research on various aspects of leprosy, national and international efforts to control and finally eradicate the disease have been intensified as never before. Leprosy is one among the six diseases identified as priority global health problem by World Health Organisation (W.H.O.). The W.H.O. has launched two massive research programmes: IMMLEP and THELEP under the Tropical Disease Research (TDR) Project. Following the adoption of resolution, "W.H.A. 44.9", on the elimination of leprosy as a public health problem by year 2000 in the World Health Assembly in May 1991, the increased political and professional commitment in several
countries to attain the goal have been phenomenal (WHO 1993). Hence, it has been given a top priority by Indian Government as well.

The availability of effective anti-leprosy therapy, assurance of complete cure, and restoration to near normal appearance have resulted in a tremendous revolution in all facets of fight against leprosy scourge. Leprosy, although medically curable, awaits a cure for its social stigma. Over the centuries, leprosy has invariably been wrongly believed to be infectious and incurable.

SPECTRUM

Immunity in leprosy is both innate (or "causal") and acquired (or "consequential"). However, once immunity is overpowered and infection is established, a spectrum of humoral and cell mediated immune (CMI) responses are manifested. A five group classification system (Ridley and Jopling 1966) recognises two polar forms - Lepromatous and Tuberculoid; and three intermediate borderline groups of leprosy patients as described in Table 1.

Imposed upon this spectrum are the reactional states, which are acute, episodic inflammatory reactions, occurring during the natural course of the disease. The type-I reversal reaction occurring in BT, BB, and BL patients may be followed by worsening or improvement of the immune responsiveness temporarily and may not be reflected in the antigen induced 48 hour skin-test reactivity (Laal et. al. 1987). The pathogenesis of type-II or erythema nodosum leprosum (ENL) reaction associated with polar LL and BL leprosy (Ridley 1969) is enigmatic, and is thought to be due to immune complex deposition in the lesions and/or increase in CMI (Tyagi et. al. 1990).
<table>
<thead>
<tr>
<th>Clinical, Histologic and Immunologic Features</th>
<th>Polar Tuberculosis (PT)</th>
<th>Borderline with Tuberculous Features (BT)</th>
<th>Borderline (BB)</th>
<th>Borderline with Lepromatous Features (LL)</th>
<th>Polar Lepromatous (PLL)</th>
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<tbody>
<tr>
<td><strong>Skin Lesions</strong></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>Intermediate between BT and BL</td>
<td>&quot;Inverted saucer&quot; characteristic but not common. LL type nodules ill-defined plaque with an occasional sharp margin</td>
<td>Ill-defined nodules, generalized diffuse infiltration, or macules like papules, symmetrical, border facts and eyebrow stippling</td>
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<tr>
<td><strong>Nerve lesions</strong></td>
<td>Skin lesions anesthesia early, nerve trunk palsy</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 palsy variable, symmetrical distal anesthesia</td>
</tr>
<tr>
<td><strong>Lepromin skin test</strong></td>
<td>Positive</td>
<td>Usual positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Histology organisms</strong></td>
<td>Rare less than 1 per 100 oil emersion field</td>
<td>Rare to 1 per 10 oil emersion field, seen in nerves</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 emersion field</td>
</tr>
<tr>
<td><strong>Lymphocytes</strong></td>
<td>Present, dense peripheral infiltration about granulomas; infiltration into epidermis</td>
<td>Present, peripheral infiltration about granulomas, variable epithelial infiltration</td>
<td>Typically lymphocytic</td>
<td>Present moderately dense and in the same distribution as macrophages</td>
<td>Sparse diffuse or in focal distribution</td>
</tr>
<tr>
<td><strong>Macrophage differentiation</strong></td>
<td>Epithelioid</td>
<td>Epithelioid</td>
<td>Epithelioid</td>
<td>Usually uninfected, epithelioid foci may be present, may show foamy change</td>
<td>Foamy, may be uninfected in early lesions</td>
</tr>
<tr>
<td><strong>Langerhans giant cells</strong></td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Immunoperoxidase studies:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes Helper: Suppressor Ratio</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>Suppressor: Cytotoxic phenotype</td>
<td>As in BT</td>
<td>Restricted to the Lymphoblastic mantle and epithelial tubercle</td>
<td>Usually in LL; rarely as BT</td>
<td>Admixed with Macrophages</td>
<td></td>
</tr>
<tr>
<td>Helper Inducer phenotype</td>
<td>As in BT</td>
<td>Admixed with epithelioid cells and in lymphocytic mantle</td>
<td>As in LL</td>
<td>Admixed with Macrophages</td>
<td></td>
</tr>
<tr>
<td><strong>HLA-DR:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Heavy staining</td>
<td>Heavy staining</td>
<td>Heavy Staining</td>
<td>Heavy Staining</td>
<td>Heavy Staining</td>
</tr>
<tr>
<td>Macrophages</td>
<td>Less in TT than in LL</td>
<td>Variable results reported</td>
<td>Usually absent</td>
<td>Heavy Staining</td>
<td>Heavy Staining</td>
</tr>
<tr>
<td>Keratinocytes</td>
<td>Strongly expressed</td>
<td>Increased number</td>
<td>Some increase</td>
<td>Some increase but usually normal</td>
<td>Unusual</td>
</tr>
<tr>
<td>Langerhans cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin 2 positive cells</td>
<td>1 in 200 cells stain positively</td>
<td></td>
<td></td>
<td>1 in 3000 cells stain positively</td>
<td></td>
</tr>
</tbody>
</table>

(Adapted from Jordan et al. 1991)
AETIOLOGICAL DETERMINANTS

The primary aetiological determinant of leprosy is an acid-fast, alcohol-fast, Gram-positive *Mycobacterium leprae*, discovered way back in 1874 by Dr. Gerhard Armaur Hansen. Although this mycobacterium remains secretive about its in-vitro cultivation capacity, it can be successfully inoculated in mice and its prolific growth can be obtained in the nine-banded armadillo (*Dasypus novemcinctus*). Since, *M. leprae* prefers to grow in tissues at temperature around 31 to 37°C, the lesions are mainly found in the skin, the cutaneous nerves and subcutaneously placed nerve trunks, the testes (leading to sterility in some males), mucous membrane of the upper respiratory tract, mouth and anterior aspect of the eye, causing lepromatous keratitis, episclerites, iridocyclitis, anaesthesia of cornea, corneal ulcer, and paralysis of orbicularis occuli muscles causing lagophthalmos and impaired nasolacrimal drainage (Job et. al. 1991). *M. leprae* can remain viable outside the human body for 7 to 46 days and has an overall mean generation time range of 18 to 42 days in contrast to 20 hours doubling time of *M. tuberculosis* (Bryceson and Pfaltzgraff 1990).

The other aetiological factors include immunity of the host which is determined by various inherited and acquired factors and also the environmental factors that may either be conducive to the parasite or to the host (Koticha 1990).

TRANSMISSION AND DIAGNOSIS

The mode of transmission of leprosy is believed to be through the nose or through prolonged skin contact with infectious patients. However, about 95 %
of the people have innate immunity against leprosy and do not develop the disease even if they mix freely or live with the patients. Moreover, as many as 80% have localised (paucibacillary) type leprosy and cannot spread the infection (Koticha 1990). Since the number of persons with sub-clinical infection in any area are far more than those who manifest the overt disease, it is of paramount importance to detect them for instituting proper immuno-prophylaxis or chemo-prophylaxis. The remaining 5% develop indeterminate lesions. Of these, in about 75% of persons there is spontaneous healing. However, a small majority of these lesions may develop into any of the established types.

Leprosy, a slowly progressive disease, has an incubation period of 3 to 30 years (Job et. al. 1991). Since the manifestations of leprosy are mainly in the skin and peripheral nerves and are easily accessible to careful physical examinations, the distribution and appearance of the disease is readily perceived and studied. There are four cardinal clinical signs of leprosy or Hanseniasis (Pfaltzgraff and Bryceson 1985): (i) hypopigmented or erythematous patch(es) in the skin, (ii) impairment of sensation in an area of the skin i.e. anaesthetic lesions, (iii) thickened peripheral nerves, and (iv) presence of noncultivable acid-fast bacilli (AFB) in the skin. Leprosy can also be immunodiagnosed serologically by employing sensitive techniques like fluorescent leprosy antibody absorption test (FLA-ABS) (Abe et. al 1976), radio-immunoassay (RIA) (Harboe et. al 1987) and ELISA for PGL-1. Molecular biology based diagnostic techniques like dot blot hybridisation with nucleic acid probes involving repetitive sequences (Clark-Curtiss and Docherty 1989), and Polymerase chain reaction (PCR) with *M. leprae* groe EL gene (Woods and Cole 1989; Plikaytis et. al. 1990), or 36 KD gene (Hartskeel et. al. 1989,) have also emerged.
Presently, the three most active antileprosy drugs available are Dapsone, Clofazimine and Rifampicin. As recommended by the WHO study group (WHO 1982) the multi-bacillary patients should be treated with a monthly dose of Rifampicin (600 mg) and Clofazimine (300 mg) together with daily dose of Dapsone (100 mg) and Clofazimine (50 mg.) for self administration. All paucibacillary patients are treated with a monthly dose of 600 mg Rifampicin and daily self administration of 100 mg Dapsone.

Immunotherapy implies immunological intervention in immunodeficient leprosy patients with established, clinical disease. Immunoprophylaxis, on the other hand, implies procedures utilised to induce protective immunity in uninfected individuals. The candidate immuno-modulators or vaccines which have been considered for investigation vary from the use of killed M. leprae with cross reactive mycobacterium like BCG (Convit et. al. 1983), M.W. (Talwar et. at. 1983), ICRC bacillus (Deo et. al. 1981), or M. vaccae (Stanford et. al. 1993), M. habana (Singh N.B. et. al. 1985), components of M. leprae like delipidified cell component (Robinson and Mahadevan 1987) or cytokines like IL-2 (Kaplan et. al. 1990), γ-IFN (Kaplan et. al. 1989) or other subunit vaccines (Jacobe Jr. et. al. 1987).

However, as many as 7 to 8 years of multiple drug therapy have had no remarkable impact on the incidence of leprosy (Ottenhoff 1994). It still remains important to understand exactly what protection is, how different T-cell subsets contribute and how cytokine networks, macrophage bactericidal mechanisms etc. can influence, to reduce or eradicate leprosy.
B. LEPROSY AND CELL MEDIATED IMMUNITY

INVASION

Events immediately following the entry of *M. leprae* into the host and during the incubation period are not known. Not only do *M. leprae* have a predilection for nerves but they are the only bacteria to have the capacity to enter nerves. Whatever may be the route of entry the target is the Schwann cell. This neural predilection of *M. leprae* results in a continuous leakage of *M. leprae* into the general blood circulation (Stoner, 1979). The infected Schwann cells show decreased proliferation and multiple axonal myelination (Mukherjee et. al. 1980) and present *M. leprae* antigens in context of HLA molecules to T cells thereby leading to DTH (Dealyed Type Hypersensitivity) response and nerve damage. To establish infection in the host, *M. leprae* must be ingested by host macrophages wherein they should survive and multiply. The exuberant growth of *M. leprae* within the macrophage of LL stands in striking contrast to the paucibacillary macrophage of TT (Sengupta 1993).

PHAGOCYTOSIS AND THE MACROPHAGE

It has been stated that the capacity of macrophages to ingest *M. leprae* is normal (Rojas-Espinosa 1978). However, another mode of direct penetration through the plasma membrane of phagocytes has also been postulated as a means of entry (Birdi 1989). This mode of entry, therefore, explains the observation that some of the bacteria lie free in the cytoplasm and parasitise non-phagocytic cells like Schwann cells and Langerhan's cells. The *M. leprae* binds through its phosphoglycolipid-I (PGL-I) to complement component C3 present on the surface of monocyte derived macrophages. The
complement receptors CR1, CR3 and CR4 present on the surface of monocyte derived macrophage further aid in mediating adherence and ingestion (Schlesinger and Horwitz 1991). Once intracellular, *M. leprae* may prevent its own destruction either by interfering with the activity of lysosomal hydrolytic enzymes or by preventing phagosome-lysosome fusion (Birdi 1989). Earlier studies have reported normal lysosomal enzyme levels in peripheral blood derived macrophages of lepromatous and tuberculoid patients (Avila and Convit 1970); while later studies revealed a decrease in the enzyme levels of lepromatous patients compared to tuberculoid patients and normal individuals (Marolia and Mahadevan 1984).

It has been postulated that *M. leprae* counteracts the toxic effects of superoxide radicals in the macrophage by scavenging them with superoxide dismutase (Wheeler and Gregory 1980). The PGL-I of *M. leprae* has been shown to scavenge reactive oxygen intermediates, which may serve to prevent the bactericidal action of the oxygen radicals (Neill and Klebanoff 1988). *M. leprae* has been reported to interfere with triggering of the oxidative burst following its phagocytosis (Holtzer et. al. 1986); however, this was later contradicted (Marolia and Mahadevan 1987). The same study (Marolia and Mahadevan 1987) has further reported that the production of superoxide in response to *M. leprae* infection is reduced in macrophages from leprosy patients but not in macrophages from normal individuals. Following the intracellular survival of *M. leprae*, macrophage metabolism gets affected, as indicated by impaired protein synthesis (Birdi et. al. 1979). The membrane alterations induced by viable *M. leprae* are reflected in the down regulation of Fc receptor, Con A receptor and HLA-DR antigen expression (Birdi et. al. 1983). The down-regulation of these receptors leads to reduced opsonisation and directly thwarts the ability of macrophages - to present protective
antigenic determinants to T cells. A macrophage lipid ganglioside GM1, reported to increase during M. leprae infection, has been speculated to affect both macrophage and lymphocyte membranes, causing a decrease in CD 4 antigen expression (Khandke et. al. 1984; Harris et. al. 1986; Kumar et. al. 1987 and Birdy 1989).

Contrary to these reported deficiencies, several studies have pointed out that monocytes in LL are not defective but remain refractory to specific activation signals following M. leprae infection. This view has been supported by observations of normal phagocytosis and inhibition of growth of other microbes like BCG inside the macrophages derived from LL in presence of exogenous activating factors like rIFN (Desai et. al. 1989) or high concentration of tuftsin (Iyer et. al. 1990). The lipoarabinomannans (LAM) purified from M. leprae have been shown to inhibit r-IFN production and T cell proliferation (Kaplan et. al. 1987). These have been speculated to behave as potent macrophage regulatory factors and expected to be leprosy virulent factors (Watson and Britton 1993).

One of the bactericidal mechanisms responsible for the killing of M. leprae in-vivo has been the activation of antigen specific cytotoxic (Kaufmann et. al. 1986) or NK cells that kill the M. leprae laden macrophage. This results in the release of the bacteria from the infected host cell into the extracellular environment where they are rephagocytosed and killed by more activated macrophages or neutrophils. In order to explore intracellular killing of M. leprae by the resistant macrophage, attempts have been made to express recombinant M. leprae antigen specific genes that may get switched on inside the macrophage.
Recently positional cloning has been applied to identify and clone a candidate for the murine intramacrophage resistance gene Lsh/Ity/Bcg in humans (Vidal et. al 1993). The candidate gene designated Nramp (Natural resistance associated macrophage protein) encodes a polytopic membrane associated protein with similarities to eukaryotic nitrate transporters. It has been hypothesised that Nramp is located in the lysosomal membrane and confers resistance by transport of nitrate into the phagolysosome of infected macrophages, where the acidic environment promotes conversion of nitrates to toxic nitric oxide radicals. Further analysis of macrophage-expressed Nramp DNA clones has identified an additional 64 amino acids at the N-terminus encoding a proline/serine rich putative SH3 binding domain, consistent with a role for Nramp in signal transduction and/or cytoskeleton attachment (Blackwell et. al. 1994). The phenotypic expression of the Bcg gene has been reported to account for difference in antigen presentation (Denis et. al. 1988b; Hilburger and Zwilling 1994), the level of MHC class II expression and its stability (Zwilling et. al. 1987). These studies provide possible evidence that the gene regulates some key point in the pathway to priming/activation of macrophages for antimicrobial activity, and contributes as a cardinal deficit in LL leading to their specific anergy.

SUPPRESSION MECHANISMS

Macrophages have been implied as suppressor cells in a number of studies. The macrophages derived from LL patients have been shown to secrete suppressor factor(s) such as, an indomethacin-sensitive, secretory factor, and an intracellular, indomethacin-resistant factor in response to viable M. leprae infection (Salgame et. al. 1983; Birdi et. al. 1984). Monocyte-derived soluble, suppressor factor of 25 kDa molecular weight, with the ability to
Suppress IL2 synthesis in LL patients has also been reported (Sathish et. al. 1983; Nath et. al. 1984). The suppressor factors exert profound immunosuppressive effects on not only macrophage functions but also induce T suppressor (Ts) cells. The lepromin-induced Ts cells have been shown to suppress the Con A-induced proliferation selectively in a majority of lepromatous and borderline patients but not in tuberculoid leprosy patients and healthy individuals (Mehra et. al. 1980). In another study, the lepromin-induced suppression of the Con A-stimulated lymphoproliferation was reported in a majority of the tuberculoid patients but not in all of lepromatous patients. On the contrary, many of the lepromatous patients showed an enhancement in the lymphoproliferative response. (Nath et. al. 1980, 1983; Nath and Singh 1980a, 1980b). Following these divergent views on suppressor-cell activity, it was reported that M. leprae antigens suppressed T cell proliferation in response to mitogens and antigens in both lepromatous and tuberculoid patients, as well as controls never exposed to M. leprae or M. leprae endemic areas (Kaplan et. al. 1987). With the advent of monoclonal antibody technology, these Ts cells of LL were later identified to be of CD8+, CD3+, FcR+ HLA-DR+, IL 2R+, CD4+ and CD28 phenotype. Their percentage in the peripheral blood declined on treatment, suggesting that the expression of these markers correlated with the antigen load and state of activation of Ts cells in vivo (Salgame et. al., 1984). Others have also correlated their findings with the presence of increased number of T8+ cells in the LL lesions, with the helper/suppressor ratio of 1:1.8. and, an increased number of T4+ cells in the TT lesions with the helper/suppressor ratio of 5.6:1 (van Voorhis et. al. 1982, Modlin et. al. 1983a, 1983b). In a later study, Modlin et. al. (1986) found that in LL lesions, T cells of T-suppressor phenotype (9.3-) were the predominant CD8+ cells and suppressor/inducer
cells (2H4+, CD4+) represented half of the CD4+ subset. The helper/T-inducer cells (CD4+, 4B4+): suppressor/inducer cells outnumbered as, 14:1 in TT lesions compared to a ratio of 1.2:1 in peripheral blood. Whereas in LL lesions the ratio was 1.1:1 as compared to 1.9:1 in blood. The CD8+ 9.3+ T cytotoxic subset predominated as 38% in TT granulomas compared to 16% in LL. Similar findings have been obtained by other studies (Narayanan et al. 1983) which indicated the helper: suppressor ratios to be 1.92 in TT, 1.88 in LL, 0.91 in untreated LL, 1.97 in ENL in peripheral blood; and 1.65, 1.5, 0.58 and 2.72 in their lesions, respectively. The CD4+/CD8+ ratios also ranged from 1.2 to 5 in TT and from 0.2 to 1 in LL lesions. While these studies suggested the involvement of Ts cells, those carried out by Gonzalez - Amaro et. al. (1987) ascribed the unresponsiveness in LL to a defective contra-suppressor immune circuit.

IMMUNOREGULATION AT THE T CELL LEVEL

Following the inductive phase of CMI, involving macrophages, the T cells constitute the next central phase of immune response. Although phenotyping of lymphocytes in leprosy lesions has provided important information about the nature of cells in the inflammatory sites, the delineation of the T cell receptor (TCR) and the effector immune function of these cells is of great potential interest. The skin lesions of leprosy provide a focal point or a window into the immunoregulatory events involved in human immune response to infection. The detection of TCR diversity provides an insight into range and nature of antigens recognised in local immune response. Most of the work reported till recently was on T cells bearing the αβ TCR complex. Lately, however, T cells bearing γδ receptor have been documented by several groups to expand in response to mycobacterial infections. The γδ T cells have
been visualised to play a dual role. On one hand, they mount a specific immune response against pathogens and on the other hand, their reactivity to self stress proteins allows them to initiate or amplify the immune reactions localised at the site of reaction. Thus it has been suggested that the $\tau\delta$ T cells may participate in host defense at the first line against the invasion of various pathogens (Janeway et. al. 1988). Inoue et. al. (1991) showed in mice that $\tau\delta$ T cells, preferably $V_r^1/V_\delta^6$ precede $\alpha\beta$ T cells in appearance after primary infection with viable BCG. These $\tau\delta$ T cells were observed to respond to sonicated BCG lysate and PPD derived from *M. tuberculosis* but not to 65 KD hsp of *M. bovis*. However, this early response, self-surveillance hypothesis for T cells in mice was contradicted by the observations of Fujita et. al. (1993) who showed appearance of $\tau\delta$ TCR positive cells (mostly $V_\delta^1$ $V_\delta^2$ $V9^+$) later than $\alpha\beta$ TCR in the lesions following lepromin reactions. TCR $\tau\delta$ positive cells were shown to comprise 5% (0.5 to 20%) of the CD3$^+$ cells in human blood and lymphoid tissues (Groh et. al. 1989) and the $V_r^2/V_\delta^2$ chain pair occurred in most individuals on more than 70% of the circulating $\tau\delta$ T cells (Faure et. al. 1988). Modlin et. al. (1989) found that in contrast to the normal low frequency of $\tau\delta$ TCR bearing cells in lymphoid tissues, peripheral blood or normal skin, the frequency increased 5 to 8 folds in particular in granulomatous reactions in leprosy. They also observed the presence of GM-CSF in the culture supernatants of activated $\tau\delta$ T lymphocytes. They, therefore, suggested that these cells may be playing a role in the immune response by stimulating granuloma formation. Uyemura et. al. (1991) showed in both lepromin skin tests and reversal reactions that TCR $\delta^+$ lymphocytes comprised 25 to 35% of CD3$^+$ T cells in the lesions compared to 5% of CD3$^+$ cells in lesions of other forms of the disease. A ratio of $V_\delta^2 : V_\delta^1 : : 2:1$ was observed in leprosy dermal granulomas, compared to 9:1 in their peripheral.
blood, suggesting a limited and selected diversity. The innate reactivity of T cells to H 37 Ra was also shown to occur almost exclusively in Vγ9/Vδ2 cells (Band et. al. 1990). The antigen presenting cells, however, for \( \tau \delta \) are not well defined. \( \tau \delta \) T cells (with Vγ9) were also shown to respond to 1.3 kDa lectin binding protease resistant component of \( \text{M. leprae} \) in a HLA-DR restricted manner. Majority of \( \tau \delta \) T cells were suggested to proliferate in response to carbohydrate or glycolipids (Pfeffer et. al. 1990).

**SPECTRUM OF ANTIGENS RECOGNISED BY T CELLS**

\( \text{M. leprae} \) presents a complicated structure and the role of its different components in protective or pathogenic-host-interaction has been probed in several ways like the use of chromatographically separated (Ottenhoff et. al. 1986) or nitrocellulose bound SDS-PAGE resolved antigen fractions (AbouZeid 1987) to stimulate T cells. Moreover, several \( \geq 11 \) cloned \( \text{M. leprae} \) protein antigens like: 70 kDa, 65 kDa, 36 kDa, 28 kDa, 18 kDa and 12 kDa have been characterised by murine monoclonal antibodies i.e. L7, L12, F47-9, SA-1B11H, LS, MLO6, respectively (Young et. al. 1985). Among these the 70 kDa, 65 kDa and 18 kDa have shown amino acid sequence homology with highly conserved hsp of other prokaryotes and eukaryotes (Jindal et. al. 1988; Shinnick, et.al. 1988; Mc Kenzie et. al. 1991 and Garsia et. al. 1989). It was, therefore, proposed that because of elevated expression of bacterial hsp within the stressful hostile environment of APCs (Buchmeier et. al. 1990), these protein antigens might represent the major targets of CMI response (Young and Elliot 1989). The crossreactivity between host and pathogen derived hsp, undoubtedly has the potential to initiate autoreactivity in the host (Van Eden et. al. 1988; Elias et. al. 1990). The 65 kDa antigen surprisingly shared its T cell epitopes with a HLA-DR2 peptide also
(Anderson et. al. 1988). Nath et. al. (1990) isolated a fusion protein (LSR-2) which appeared to be of 10 kDa size and a potent stimulator of T cells in TT patients. The 30 to 31 kDa proteins have been characterised to be major secreted antigens of \textit{M. leprae} and \textit{M. tuberculosis} and correspond to the antigen 85 complex. This complex has the ability to bind to fibronectin and therefore plays an important role in ingestion of \textit{M. leprae} into host cells (Thole et. al. 1992).

Specific T cell epitopes were mapped on proteins of 65 kDa (van Schooten et. al. 1988), 38 kDa (Anderson and Hansen 1989), 19 kDa (Ashbridge et. al. 1990) and 18 kDa (Dockrell et. al. 1989) using recombinant technology. However, since the expression of \textit{M. leprae} genes cloned in recombinant expression vectors were selected with antibodies raised in non-human hosts, the antigens selected appeared to be more relevant to antibody responses than to the T cell mediated mechanisms which are fundamental to leprosy. Therefore, the T and B cell epitopes were further studied without preselecting them with antibodies (Abou-Zied 1987). Using biochemically purified antigens, T cell recognition of 36 kDa (Van Schooten et. al. 1988), 35 kDa, 70 kDa, and secreted 32 kDa (Janson et. al. 1991) were also reported. Employing highly purified \textit{M. leprae} cell wall-associated proteins, T cell reactivity was observed to protein fractions of 7, 16 and 28 kDa antigens of \textit{M. leprae} (Mehra et. al. 1989).

An overlap was observed between humoral and cellular response of paucibacillary (TT, BT) patients against the nitrocellulose bound \textit{M. leprae} or BCG antigens, however, the two responses remained focussed primarily towards different molecular ranges i.e., humoral >40 kDa and cellular < 40 kDa (Ottenhoff et. al. 1989). In multibacillary (BL,LL) patients, the humoral and cellular responses were often non overlapping. The TT patients
recognised and mounted a stronger immune response to low molecular weight antigens, 10-25kDa of *M. leprae* and >70 kDa antigens of BCG. However, the LL responded to higher range (>150 kDa) antigens. The household contacts showed relatively flat profile. Sera obtained from LL recognised 21 bands of 97-14 kDa especially 28 and 65 kDa proteins of SDS-PAGE resolved sonic extract of *M. leprae*. Whereas contacts recognised 18 bands, especially the 16 kDa in the same interval of 97-14 kDa. Similarly, normals recognised 19 bands, especially 18 kDa protein (Rodriguez et. al. 1993). This experimental approach was further extended to bidimensional SDS-PAGE resolution of *M. leprae* antigens (Guile et. al. 1992). In another molecular study of the T cell repertoire in familial contacts and leprosy patients, the response to 12-19 kDa and 17-15 kDa nitrocellulose bound *M. leprae/M. tuberculosis* sonic extract fractions were observed to be high in tuberculoid than in lepromatous families. The response, however, to 65 kDa fraction was found more frequent in both lepromatous and familial contacts than tuberculoid cases (Samperio et. al. 1989).

Basically the total spectrum of antigens recognised by T cells remains wide. There is as yet no evidence for antigens that are exclusively recognised by patients or by healthy individuals so far (Ottenhoff. 1994). Few vital aspects still remain unresolved where *M. leprae* antigens that trigger TT cells and thus protect them from developing LL and antigens that can induce protective immunity, avoiding tuberculoid pathology in healthy contacts, are demarcated.

**CYTOKINE PROFILE**

The effective mechanisms of the immune response manifest themselves via a complicated interplay of various cytokines. The antigen specific or non
specific factors released by immune cells residing in the leprosy granulomas can easily modulate the antigen specific interactions in the immediate vicinity. (Romagnani 1994). The main antigen non-specific factors can be one of the interleukins or interferons or the B cell stimulating factor. The antigen-specific factors can behave as either helper or suppressive type and substitute for direct cell to cell interaction. The TCR-activated signal transduction pathways contribute to the induction and activation of several lymphokine gene regulators like NF-KB, AP-1, AP-3, NF-AT, and Octamer binding factors (Gauchat 1991). Further proliferation of resting (Go blocked) T cells requires two inductive signals i.e. the antigen and IL-2, sequentially (Smith 1981). The post transcriptional regulation of cytokine expression is mediated through the presence of AUUUA repetitive elements in the 3' non-coding regions of mRNA thereby leading to their instability (Shaw and Kamen, 1986). T cell subsets were defined functionally on the basis of their lymphokine secretion pattern in mouse (Mosmann and Coffman 1989) and more recently confirmed in humans also (Romagnani 1991, 1992, 1994). The TH1 cells produce IL-2, γ-IFN, TNF-β and lymphotoxin, but little IL-4 and IL-5 and are primarily involved in mediating cell mediated responses against intracellular parasites and bacteria. TH2 cells serve as helper cells for antibody production and preferentially produce IL-4, IL-5 and IL-6, IL-9, IL-10 and IL-13 but little IL-2 or γ-IFN. The TH1 are responsible for both humoral and cell mediated immune responses, macrophage activation, antibody dependent cellular cytotoxicity and delayed type hypersensitivity reactions whereas TH2 provide help for humoral immune responses. In the absence of clear 'polarising' signals, the TH0 give rise to a less differentiated cytokine profile. γ-IFN selectively inhibited proliferation of TH2 (Romagnani 1992) whereas IL-4 induced their proliferation. A similar
polarised heterogeneity also existed for CD8+ T cells (Pabard et al. 1988). Correspondingly, two subsets of CD8+ clones specific for M. leprae antigens were described. CD8+ cytotoxic clones produced γ-IFN and IL-10 but not IL-4 whereas CD8+ suppressor clones produced substantial amounts of IL-4 (Salgame et al. 1991). Yamamura et al. (1991) revealed striking differences in cytokine mRNA profiles in lesions using PCR techniques. While mRNAs for IL-2, γ-IFN, LT, IL-6, IL-1, GM-CSF, TNF-α and TGF-β were abundant in tuberculoid lesions, IL-4, IL-5 and IL-10 were found in greater amounts in LL lesions. The mRNAs, IL-3 and IL-8 were not observed in both type of lesions. Interestingly, these patterns of cytokine mRNAs reflected the polarity of T subsets TH1 and TH2 in TT and LL respectively i.e. the resistant and susceptible forms of the disease. The PPD or mycobacterial 65 kDa hsp specific CD4+ T clones from healthy donors were shown to preferentially produce TH1 cytokines (Del Prete et al. 1991 and Haanen et al. 1991).

The induction of TH1 cells was specific to mycobacteria because the other antigens induce T cells with much lower γ-IFN/IL-4 ratios (Haanen et al. 1991). Mutis et al. (1993) reported that the peripheral blood derived Ts cells from LL released significantly more γ-IFN than IL-4, although they did produce more IL-4 than TT (Haanen et al. 1991). This suggested that IL-4 did not play a major role in suppression. However, the mechanism of suppression with respect to IL-4 remained confusing.

Sieling et al. (1993) hypothesised that IL-4 was produced by CD8+ Type 2 cells in LL lesions that resulted in further clonal expansion of this subset. The immunosuppressive action of IL4 on T cells and macrophages facilitated growth of M. leprae, the released antigen then could provide a strong
stimulus for IL-10 secretion. The utility of IL-10 to suppress M. leprae specific T cell responses could result in synergism with IL-4 to suppress CD4+ T cell proliferation and production of τ-IFN, perpetuating local immunosuppression. IL-10 and IL-4 were observed to execute opposing effects on M. leprae induced cytokine synthesis by macrophages. IL-10 produced in response to M. leprae inhibited secretion of TNF-α and GM-CSF, two cytokines with antimycobacterial effects, but that IL-4 regulated this effect by inhibition of IL-10 release.

The early predominance of, or subsequent shifting to TH1 type responses thus contributes to DTH, clearance of M. leprae by activated macrophages and protective immunity and in some case immunopathology. In contrast, predominance of and/or shifting to TH2 like response leads to chronicity of infection and because of the activity of IL-4 and IL-10, to specific immunosuppression (Romagmani 1994). Despite the extensive knowledge of the various cytokine patterns in leprosy, the diversity of antigen that stimulate these respectively has not been examined. The delineation of those cytokines which can prevent nonresponsiveness and turn on protective TH1 cells but not pathogenic has also not been described.

Nevertheless, attempts are being made to reverse the M. leprae specific anergy by triggering the T cells of LL in-vitro by low doses of exogeneous cytokines such as IL2, (Ottenhoff 1989), injection of τ-IFN (Nathan N. 1986), PPD (Kaplan G 1986), injection of both IL-2 and τ-IFN together in the LL lesions, (Shinde et.al. 1993), in addition to use of dendritic cells as APCs (Nath 1986) and preincubation of cells for 48 hr (Mohagheghpour 1987).

The humoral responses seem to play little role in protection in leprosy even though the leprosy patients develop antibodies to specific as well as
cross reactive \textit{M. leprae} antigens. (Wemambu et. al. 1969; Yumnam et. al. 1977; Kelkar et. al. 1979; Lunder et. al. 1979; Sengupta et. al. 1979; Melsom et. al. 1982b; Furukawa et. al. 1984; George et.al.1986).

C. LEPROMSY AND GENETIC PREDISPOSITION

The possible role of host genetic background in resistance and susceptibility to leprosy is supported by several lines of evidence. One fact that emphasises the genetic contribution to the expression of leprosy is the large individual variation in the response to \textit{M. leprae} which is correlated with the cellular immune responses of the patient. The variable incidence rate of leprosy among certain ethnic groups (Aycock 1940), disappearance of endemic foci in developed countries (Badger 1951, Fieldman 1976, Leiker 1977) despite the immigration of patients from other endemic areas of the world and the restriction of the disease to certain familial clusters living in endemic areas (Shield et.al 1987) represents a changing genetic influence on susceptibility.

FAMILY CLUSTERING AND TWIN STUDIES

In humans, approaches to the genetic susceptibility to leprosy have progressed along two complementary lines - on one line are studies of familial aggregation, including various investigations as twin studies; on the other line are the relationships between leprosy and genetic markers, at either the population or the familial level (Abel et.al. 1989). The concordance rate for leprosy was shown to be significantly higher among monozygotic twins (60-85\%) than among dizygotic twins (15-20\%) (Chakravarti and Vogel 1973, Mohamed Ali and Ramanujan 1966).
HLA- ASSOCIATIONS AND OTHER GENETIC MARKER STUDIES

The linkage disequilibrium leading to the prevalence of certain combinations of alleles of different loci in one of the most polymorphic genetic systems i.e the HLA has not only borne functional importance but has also been exploited for the demonstration of susceptibility to mycobacterial infections. Genetic studies of leprosy have failed to disclose any significant association between HLA type and susceptibility to disease. However, an association between HLA and the form of disease acquired have been established. Several studies on class I antigens (HLA-A,-B and -C) showed weak associations confined only to the populations tested and were usually not reproducible. This implied that class I specificities were not the right markers for HLA encoded Ir/Is genes (Ottenhoff and de Vries 1987, van Eden and de Vries 1984).

More convincing and consistent results were obtained when HLA class II antigens were studied (Ottenhoff and de Vries 1987). Population and family studies have shown that tuberculoid leprosy was associated with HLA-DR 2 in India (de Vries et. al. 1980), in Japan (Miyamaga et al 1981) and in Thailand (Schanff, et al 1985) or with HLA-DR 3 in Surinam (Ottenhoff and de Vries 1987); while LL was associated with HLA-DQW 1 in Venezuela (Ottenhoff et al 1984, van Eden et. al. 1985). The family segregation analysis to test for linkage between HLA and disease phenotype showed a non-random allocation of HLA haplotype among TT children of nonafflicted parents and parents with LL and among BL/LL children of LL parents. The HLA-DR3 was inherited preferentially by children with polar TT than LL and HLA-MT 1 (DQ 1) by children with LL (Fine et. al. 1979, van Eden et. al. 1980, Blackwell 1988). Therefore, it was interpreted that DR3 may induce a strong T cell
response and predispose to TT while DQ 1 instead may induce a state of nonresponsiveness, perhaps through induction of *M. lepraee* specific suppressor cells, and thus predispose to LL. This has been supported by the observation of a Mendelian form of inheritance of the macrophage in-vitro responses to viable *M. leprae* which include down regulation of Fc receptors thereby suggesting that the macrophage defect could be an innate one (Birdi et al 1983).

Functional in-vitro studies have confirmed the role of DR molecules in presentation of *M. leprae* antigens to specific Th clones with functional polymorphism mapped down to a single amino acid difference in the DRβ1 chain (Ottenhoff et al 1986). Numerous other genetic marker studies were conducted to identify pleiotropic effects of hypothetical genes like human blood groups ABO, Rh, G-6-PD deficiency, etc. (Abel et al 1989, Smith 1979), but none of them, however, were found to be consistently associated with a particular host response.

Since all these associations were not strong and showed heterogeneity, it was suggested that the primary disease genes may be at a locus closely linked with HLA-DR rather than HLA-DR locus itself. Alternatively, non HLA genes were suggested to contribute to host immune response to *M. leprae*. The candidate non-HLA genes in leprosy severity were presumed to be TCR genes or the human homologue of the murine Bcg gene which controls resistance or susceptibility to a range of intracellular parasites including *M. lepraemurium* (Jazwinska and Serjeantson 1988). Genes encoding both α and β chains of human TCR distinguishable by restriction fragment length polymorphisms (RFLP) (Seboun et al. 1989) have served as markers for TCR α and β chain haplotyping in families. The TCR gene subfamilies VB 6.1 through VB 6.4 were reported to be overexpressed in TT and not LL.
lesions (Wang et al. 1993). However, earlier genetic studies using HLA-DR, DQ DNA genotyping and TCR RFLP analysis in leprosy patients revealed no association with susceptibility to leprosy in humans. Further, the TCR $\alpha$, $\beta$ and $\gamma$ gene RFLPs revealed no germline defects or major clonal T cell expansion in either LL or TT patients (Jazwinska and Serjeantson 1988).

ANIMAL MODEL AND "BCG" GENE (Non-HLA Associations)

While most of the information regarding the influence of host genes on the outcome of mycobacterial infection in humans has been circumstantial, animal model, where the influence of environmental factors can be controlled, provide direct evidence for this type of regulation (Skamene 1989). Several groups have established that natural resistance to infection with intracellular parasites such as Salmonella typhimurium, M. bovis, M. leprae and Leishmania donovani is controlled in the mouse by the pleiotropic expression of a single dominant autosomal gene given the appellations, Bcg (Gros et al. 1981, Skamene et al. 1984), Ity (Plant and Glynn 1976), and Lsh (Bradley 1977). Linkage analysis in segregating backcross progeny and recombinant inbred mice (Malo et al. 1993) have established that this locus maps to the proximal portion of mouse chromosome 1 whose high resolution genetic map has revealed the gene order and the interlocus distances (in CM):Centromere-Co13a1-(8.8)-Cryg-(2.6)$\lambda$Mm1C163-(1.6)-Fn-1-(2.0)-Tp-1-(1.0)-D1Mcg105-(0.1)$\lambda$Mm1C165/Vil/Bcg-(0.2)$\lambda$Mm1C136-(0.3)-Des/D1Mit7-(0.1)-Inha-(2.8)$\lambda$Mm1C153-(2.4)$\lambda$Mm1C156-(1.2)-Pax-3-(5.6)-Akp-3-(0.8)-Acrg-(2.0)-Sag-(0.5)-Co16a3. (Malo et al. 1993). This 35 cM fragment around the murine Bcg locus has been conserved syntenically onto the telomeric end of human chromosome 2q (Fig 1) (Schur et al 1990) and bovine chromosome 8 (Skow et al. 1987). Ever since, several experiments have
Fig. 1: Human chromosome 2, depicting the presence of different genes in the region 2q 31-35. (Adopted from UK DNA Probe Bank Catalogue, Fourth edition, Nov. 1993).
been conducted in pursuit of the human counterpart of the murine Bcg gene, besides defining the murine Bcg gene product. The recent cloning of a candidate gene, designated Nramp (natural resistance-associated macrophage protein), that controls resistance to intracellular parasites, indicated that resistance was controlled by a transmembrane, transport-like protein, with multiple transmembrane domains. A single amino acid substitution in the second transmembrane domain appeared to account for the differences in Bcgr (resistant allelic form) versus Bcgs (susceptible allelic form) mice (Vidal et al. 1993). Despite the cloning of this gene, the exact mechanism of genetic resistance, however, remains undiscovered. The phenotypic expression of the gene was reported to account for differences in TNF-α production, the production of reactive oxygen and nitrogen intermediates, differences in antigen presentation, the level of MHC class II expression and its persistence (Zwilling et al. 1987; Denis et al. 1988a; Denis et al. 1988b; Denis et al. 1988c; Roach et al. 1991; Hilburger and Zwilling 1994), selection of TH1 like subsets (Kramnik I et al. 1994) and development of suppressor cell precursors in the early phase of infection (Gosselin et al. 1994). However, others did not find functional differences between macrophage populations from resistant and susceptible mice (Appelberg and Sarmento 1990; Brett and Butler 1988).

**HUNT FOR "Bcg" HOMOLOGUE IN HUMANS AND CURRENT STATUS**

The presence of a gene that could determine susceptibility to leprosy and be located on human chromosome 2q was investigated recently. Since in the mouse, the Ity locus was mapped to the short arm of chromosome 1 (Plant et al. 1982), together with the gene family encoding the r-crystallins of the mouse eye lens (Len-1 or cryg) (Skow 1982) which are syntenically conserved on
human chromosome 2q, the linkage disequilibrium between Cryg RFLPs and susceptibility to infectious diseases was analysed (Jazwinska and Serjeantson 1988; Blackwell et. al. 1991). However, these RFLP patterns did not associate with leprosy susceptibility in humans. Similarly, in another study on French polynesian population, a set of 8 markers on human chromosome 2q (CRYG P1, FN, TNP1, VIL, DES, INH, PAX3 and UGT1A1) has depicted no evidence for linkage with susceptibility to leprosy (Levee et. al. 1994). Another linkage analysis study of a set of 9 markers in 2q33-q37 region including FN, TNP1, VIL and Des on Pakistani and Brazilian families, also concluded no association between these markers and susceptibility (Shaw et. al. 1993).

Apparently, predisposition to leprosy appears to be of a multifactorial nature with at least two groups of genes participating in the control of the disease: non-HLA linked autosomal recessive genes (Abel and Dennenias 1988) which determine susceptibility to leprosy per-se and HLA linked genes which are implicated in the spectral clinical manifestations of the disease (Levee et.al. 1994). With the identification and cloning of Nramp gene (Vidal et. al. 1993) and the advent of modern molecular biology techniques, an increasing quantum of hereditary diseases are becoming amenable to diagnosis and the responsible genes are being located and identified.

The DNA sequence differences determine both the pathological and benign genetic variations. The human genome contains a large number of different tandemly repeated sequence families which include three major classical satellites I, II and III (Prosser et. al. 1986); minisatellites consisting of a hypervariable "Core sequence" of 17-35 bp, present as a variable number of tandem repeats (VNTR) (Nakamura et. al. 1987), giving rise to a highly
polymorphic or hypervariable region HVR (Higgs 1981); microsatellites consisting of tandem arrays of simple repeat sequences (1-66 bp long) including simple sequence families of repeating mono-, di-, tri-, and tetra- nucleotides (Wood and Langlois 1991).

The identification of sequence tagged repeats (STR) may be identified along with their genetic and physical mapping utility, have given these the property of useful sequence tagged sites (STS). The tandem reiteral, regardless of the core sequence motif, predisposing to variation, can be easily typed by PCR and precisely resolved to single bases by polyacrylamide sequencing gels. The human chromosomal region 2q32-q37, the importance of which has been highlighted previously, also contains several polymorphic elements which can be typed as genetic markers and analysed for association with susceptibility to leprosy. To exemplify a few, a polymorphic (AAC)₉ repeat (STR) located at base 3021 of the human β crystallin gene (Hearne and Todd 1991) which exists in at least three allelic forms have been PCR typed as 191 bp, 188 bp, 182 bp long amplicons. The cytotoxic T lymphocyte CTLA4 gene, in the same chromosomal region, also harbours another polymorphic (AT)n repeat at 642 bp of exon 3 (Polymeropoulos 1991). Using specific primers in genomic PCR the alleles have been identified to be of 130 to 92 bp length range. In addition, the collagen III alpha I chain gene col 3A contains a VNTR and can be geno-typed to as one of the 312 bp, 284 bp, 270 bp, 250 bp, 236 bp allelic forms (Lee 1991). All these different allelic forms have been shown to follow Mendelian patterns of inheritance in three generation families.

Identification of single base pair mutations by the above mentioned PCR genotyping has already paid dividends in Ehlers–Danlos syndrome (Lee et al. 1991) among several others. No attempts, however, have been made to
our knowledge, to study the PCR based geno-typing of allelic variants confined to the relevant region of human chromosome number 2 to explore an association, if any, with susceptibility to leprosy. This interested us to investigate variations in the PCR genotyped allelomorphs of Col 3A and CTLA4 and Cryg1 genes located on chromosome 2q31-35. We further explored the possibility of cosegregation of these defined allelic variants with any of the phenotypic manifestations of leprosy, basically the cell mediated immune response, gauged in terms of (a) in-vitro lymphocyte transformations, (b) enumeration of CD4 : CD8 and memory : naive cells and (c) the mRNA levels for TH1 or TH2 type cytokines, in the peripheral blood.