SUMMARY AND CONCLUSIONS
Leprosy remains a major health problem throughout the world. It displays a spectrum of clinical manifestations correlating with the level of cell-mediated immunity in the patients (Myrvang et al. 1973). Lepromatous leprosy (LL) patients show T-cell anergy to *M. leprae* while tuberculoid leprosy (TT) patients mount strong cell-mediated immune responses to *M. leprae* antigens. Several reasons have been put forth to explain the T-cell anergy prevalent in LL patients. These include: deficiency of cytokine production (Sieling et al. 1993); presence of suppressor cells (Mehra et al. 1980; Nath et al. 1984; Modlin et al. 1986); paucity of *M. leprae* reactive T cells (Lim et al. 1974); and defective macrophage functions (Nathan et al. 1986).

Racial differences, family clustering and early twin studies suggested a genetic component to the susceptibility of the disease (Chakravarti and Vogel 1973; Abel et al. 1989). Although associations between disease phenotype and particular HLA class I and II antigens have been observed at the population level (de Vries et al. 1980; Ottenhoff and de Vries 1987), it has been difficult to demonstrate linkage between susceptibility to disease per-se or particular disease phenotypes (Jazwinska and Serjeantson 1988; Blackwell et al. 1991; Shaw et al. 1993; Levee et al. 1994).

According to the two locus model (Abel et al. 1989), the major gene controlling susceptibility to leprosy per-se has been suggested to be an analogue of the murine 'Bcg' gene located on the long arm of human chromosome 2 (Shaw et al. 1993). Whereas, secondary genes like HLA or HLA linked loci have been implicated in regulating the progression of leprosy across its spectrum.

The aim of the present study was to categorise various normal, healthy contacts and leprosy patients as responders or non-responders to mycobacterial
antigens by conducting lymphocyte transformation tests; enumerate CD4, CD8, CD45RO and CD45RA cells flow cytometrically and correlate with cytokine expression using RT-PCR, if possible. Further, the major objective of the study was to use the potential power of PCR and SSLP analysis in various subjects to test for possible correlations, if any, between the disease phenotype or the cell mediated immune status and the genetic markers -COL3A, CTLA4 and CRYG1 located on 2q31-q35.

The predominant finding of the present investigation drew attention to the dichotomy in CMI perturbations that occur in paucibacillary and multibacillary forms of leprosy (Sengupta 1993). The healthy contacts and stable BT subjects displayed the expected integrity of T cell proliferative response to PHA as well as other mycobacterial antigens (BCG, PPD and WCFE) in contrast to the hyporesponsive/anergic lepromatous leprosy (BL, LL) patients. However, the multibacillary patients included in this study showed a generalised low response to other mycobacterial antigens, BCG and PPD as well, thereby indicating that their anergy was not specific to M. leprae but even extended to other crossreactive mycobacteria.

In this study, an attempt was made to determine the phenotype of lymphocyte populations prevalent in vivo in circulation together with functional studies in order to grade CMI across the spectrum in leprosy.

The freshly isolated PBLs were immunostained with monoclonal antibodies OKT4, OKT8, 2H4 and UCHL1 and enumerated flow cytometrically. In accordance to other reports (Narayanan et. al. 1983), the helper: suppressor ratios in the peripheral blood apparently declined from healthy contact and tuberculoid to lepromatous forms. In this study the CD4:CD8 ratio was apparently high in HC responders to WCFE but not to PPD. Conversely, the
proportion was high in LL responders to PPD as compared to responders to WCFE, possibly resulting from the predominance of CD4 (T_H) cells in the responders and the predominance of CD8 (T_{s/c}) in the non responders. Further, the present study showed that the ratio of memory (UCHLI^+) to naive (2H4^+) cells were high in the immunologically more active, tuberculoid form of leprosy and HC, responsive to both WCFE and PPD. The same was observed in LL responders to PPD. However, the memory to naive ratio was observed high in LL non-responders to WCFE but low in LL responders. The perturbation in the balance of these reciprocal immunoregulatory subsets observed in this study suggest the need to study further in future the cellular events which control and modulate the differential expression of CD45 R isoforms in leprosy patients and their familial contacts.

An attempt was made in this study to examine γ-IFN expression by RT-PCR in a few representative cases in an effort to combine phenotypic determinations with analysis of cytokine profiles. The γ-IFN expression appeared to be slightly higher in tuberculoid patients than in lepromatous individuals.

The diverse observations in CMI were further correlated with the inherent heterogeneity in the allelomorphs of COL3A, CTLA4 and CRYG1 located at chromosome 2q31-q35 region. Although CRYG1 appeared to be uninformative, the experiments carried out with the other two genetic markers COL3A and CTLA4 turned out to be fruitful. The COL3A amplified products when analysed by PAGE showed 5 allelomorphs, ranging from 236 to 312 bp size. The CTLA4 amplified products of 84 to 120 bp size representing 8 alleles when analyzed on denaturing PAGE, displayed simple sequence length polymorphism (SSLP). Notably the 250 bp allelomorph of COL3A and 84 bp allelomorph of CTLA4 were found in predominance in both normal plus healthy contact
category as well as total leprosy patients. Further, the 236, 284 and 312 bp alleles of COL3A and the 98, 114 and 120 bp allelomorphs of CTLA4 were not observed in homozygosity both in normal and diseased subjects studied.

When the status of various allelomorphs of COL3A and CTLA4 were compared within the responsive/nonresponsive healthy contacts and leprosy patients it was noticed that the 270 bp COL3A homozygotes (270/270 bp) responded to WCFE. Whereas, the 312 bp COL3A heterozygotes were nonresponsive to both PPD and WCFE, irrespective of the clinical status of the cases analysed. This suggested a possible association between heterozygosity for 312 bp allele of COL3A and the nonresponder status to mycobacterial antigens in general.

Further, a comparison of the disease phenotype and the various allelomorphs of these genetic markers revealed the probable association of N+HC, paucibacillary and multibacillary forms of leprosy with 104 bp, 98 bp and 106 bp CTLA4 alleles, respectively. An absence of, COL3A allelomorphs of 236 bp in homozygous/heterozygous conditions in multibacillary leprosy patients and, 270 bp in homozygous state in N+HC category was also observed. However, the cosegregation of the disease phenotype with allelomorphs of COL3A and CTLA4 located in the long arm of chromosome 2 needs unequivocal establishment in future studies.

Nevertheless, in this study a probable cosegregation of the responder/nonresponder status was apparently conclusive. However, further extensive studies are required to find a leprosy susceptibility gene in linkage disequilibrium with the known/unknown gene markers (in addition to what has been already studied in this work) located at the chromosome 2q31-q35 region, the suggestive site for human 'Bcg' gene.