SUMMARY AND CONCLUSIONS
A focal point in the pathogenesis of leprosy is the T cell mediated impaired immune response, resulting in dynamic alterations in the clinical presentation of the disease. At one point of the spectrum, tuberculoid leprosy patients develop high levels of specific cell mediated immunity that ultimately kill and clear the bacilli in the tissues, although often with concomitant immunologic damage to the nerves. At the lepromatous pole, patients exhibit a selective unresponsiveness to antigens of *Mycobacterium leprae* and the organisms ineluctably multiply in the skin, often to extraordinary numbers (Bloom, 1986). Most studies have dealt with the role of T cell bearing αβ antigen receptors in the outcome of *M. leprae* infection. The present study was an attempt to analyse the probable association of genetic diversity in γδ T cell antigen receptors with the spectrum of immune response observed in leprosy patients. Our specific queries, after immunological categorization of leprosy patients on the basis of their response to distinct components of *M. leprae* proteins, were to find out

a) If the genetic variations at γ and δ TCR loci could be associated with the spectrum of immune response in leprosy patients or with heterogeneity of immune response in patients with the same clinical phenotype.

b) If there was any relation between the diversity of the γδ T cell repertoire in the peripheral blood and the differential *in vitro* immune response in leprosy patients.

We evaluated *in vitro* immune response of leprosy patients and healthy contacts to unfractionated (WCFE) as well as fractionated (nbCFE) antigens of *M. leprae* by lymphotransformation tests. *In vitro* cell mediated immunity to WCFE
paralleled the clinical and histopathological spectrum in leprosy patients. Unexpectedly, a sizeable number of borderline tuberculoid patients failed to respond, thereby suggesting that all paucibacillary patients do not show strong cell mediated immune response to *M. leprae* antigens. This was suggestive of the presence of hyporesponders and hyperresponders in both paucibacillary as well as multibacillary patients. However, a significant number of nonresponder subjects within each category turned into responders when their PBMCs were challenged with fractionated *M. leprae* antigens, thus ruling out the possibility of lack of *M. leprae* reactive cells as the reason for *in vitro* anergy observed in non responder patients. Interestingly, these subjects did not recognize same antigenic components of *M. leprae*. A rather broad range of antigens appeared to be seen by PBMCs from leprosy patients. This was also apparent from the comparative analysis for *in vitro* response to WCFE and nbCFE in subjects within each category. Further, we found that a significant number of paucibacillary patients did not respond to 66-45 kDa (FII) of *M. leprae* proteins, which were able to elicit proliferative responses in healthy contacts. Leprosy patients and healthy contacts were further categorized on the basis of their response to distinct groups of *M. leprae* antigens and analyzed for variations at TCR γ and δ loci.

A number of observations indicating a key role of γδ cells in the early immune response to mycobacterial antigens (Janis *et al.*, 1989; Modlin *et al.*, 1989; Born *et al.*, 1990; Tsuyuguchi *et al.*, 1991) led us to screen DNA samples from leprosy patients and healthy contacts for germline variations at TCR γ and δ loci. These loci were found polymorphic but their variations were not confined to leprosy patients only, suggesting that variations at these loci alone, did not predispose *M. leprae* infected hosts to leprosy per se. However, higher frequency
of germline variations at γ locus in multibacillary patients was suggestive of these probably acting as additional risk factors. To substantiate this hypothesis, still a larger sample number needs to be explored in future.

The finding that the paucibacillary subjects, polymorphic at Jγ did not respond in vitro to 66-45 kDa (FII) M.leprae antigens implicated a probable association between the allelic polymorphism at Jγ and in vitro anergy to these antigens in leprosy patients. The precise mechanism by which polymorphic Jγ alleles might influence the response to mycobacterial antigens remains to be elucidated.

The methylation pattern at TCR γ and δ loci was also analysed in leprosy patients and healthy contacts. These gene segments were found methylated. However, no variation was found in the methylation pattern of DNA from leprosy patients and healthy contacts, suggesting a lack of polymorphism at Msp I sites in TCR γ and δ locus in leprosy patients.

In the recent past, role of specific gene segments of rearranged TCR has been implicated in antimycobacterial reactivity in leprosy (van Schooten et al, 1992). In the present work, frequencies of rearrangements in circulating T cells, specifically of Vγ7/8 and Vγ9 were apparently low in leprosy patients as compared to healthy contacts. Moreover, within leprosy patients, these were detected less frequently in multibacillary subjects. These findings suggest a possibility of a primary genetic difference in repertoire generation in leprosy patients and an association of detectable rearrangements in circulating PBMCs with protection in in vivo conditions.
Results from the analysis of Vδ2-Jδ1 rearranged sequence amplification in DNA from leprosy patients and healthy contacts were suggestive of a probable relation between the diversity of γδ T cell repertoire in circulating PBMCs and different stages of *M. leprae* infection. Vδ2-Jδ1 amplicons of different molecular sizes were obtained. No such major variations in length of these products have been reported so far. Interestingly, Vδ2-Jδ1 amplicons of 140 bp were detected only in paucibacillary patients. Most of these subjects were undergoing type I reactions. This was implicative of the probability that cells with different rearrangements at Vδ2-Jδ1 might predominate in circulation of leprosy patients during different stages of the disease. In two leprosy patients, unexpected Vδ2-Jδ2 rearranged amplicon was detected in DNA from circulating PBMCs of leprosy patients. Extending such findings on larger sample size may prove worthwhile for gaining better insight into intricacies of immunoregulatory mechanisms.

The present study again confirms the conclusion of previous observations made in the context of HLA molecules that there can not be a single gene controlling susceptibility to leprosy per se or leprosy type. However, results from the present study are probably implicative of genetic distinction between responders and non-responders to specific groups of *M. leprae* antigens in leprosy patients, even if they manifest same clinical phenotype. Variations in the frequency of circulating cells with detectable rearrangements at TCR γ locus and diversity in the circulating γδ T cell repertoire of leprosy patients, also probably point to the genetic differences in leprosy patients.

The implications from the present piece of work are interesting for the emerging concepts regarding the function of the TCR-γ/δ and for the pathogenesis
of leprosy. Extending genetic analyses of TCR γδ on larger number of leprosy cases, particularly from multiplex families (with at least two affected sibs) and controls from nonendemic areas may probably disclose some of the secrets behind the 'central dogma' of leprosy.