SUMMARY

Tuberculosis is a chronic mycobacterial disease characterised by cellular immune reactions (Kaufmann, 1995). The present study deals with pulmonary tuberculosis (PTB) and also extra pulmonary tuberculosis like pleural effusion. This has been a major health problem for many developing and industrialised countries (Sudre *et al*, 1992). One third of the world's infected population (500 million) lives in India alone (Dollin *et al*, 1994). PTB is a prevalent disease in our study area i.e. North Eastern Hill Region. In addition to the environmental, nutritional and epidemiological factors, several lines of evidence point towards an important role of genetic factors in influencing the susceptibility/resistance to tuberculosis.

In this study, HLA class I and class II polymorphism has been studied in 120 pulmonary tuberculosis, 48 pulmonary effusive patients and 282 health controls from North Bengal region using two stage microlymphocytotoxicity technique. In addition to HLA, the antibody (IgG and IgM) levels, sub population of T and B cells were also studied to find out the pathological, clinical and immunological relevance of these serological markers to the clinical disease expression and to host MHC genes.

The present investigation revealed that a high number of patients (n = 89) were having lesions of both the lungs and 46.06% (n = 41) of these were having severe disease. 31 patients were having unilateral limited disease. No examples of unilateral extensive disease was found. Among bilateral severe diseased group 58.5% were drug failure. 34.16% of the total patients were sputum negative when the chemotherapy was initiated. This proves their position among high landers and in the low zone of tolerance.

HLA-A9 and B7 were more frequently present in PTB patients than in controls (33.3% vs 8.33%, $p < 0.01$, RR = 5.63). HLA-A2 was decreased in the patient group as compared to controls (3.3% vs 25.0%, $p < 0.01$, RR = 0.10).

HLA -DR2 was more frequently present in PTB patients than in control (36.6% vs 4.16%, $p < 0.05$, RR = 13.02). No significant correlation was observed in HLA allelic frequencies in various patient groups based on radiological lesions on the lung, though a slight higher percentage of DR2 was found in bilaterally extensive group. DR2 association was strongest among the drug failure group of patients while no such association was found in drug responder group.

Immunoglobulins IgG and IgM are known to have important clinical and biological implications. As compared to control values (IgG = 1288.6 mg/dl, IgM = 190.52 mg/dl), the level of IgG was significantly higher in PTB patients (1723 mg/dl, significant at 0.1% level), possibly suggesting that the occurrence of this antibody is related to infection or exposure to *M. tuberculosis*. The lower level of IgM in the patients (154.5 mg/dl, significant 0.1% level) may prove a significant marker. In pleural effusive patients both the levels of IgG (2188mg/dl, significant at 0.1% level) and IgM (210 mg/dl, significant at 0.1% level) were increased. IgG antibodies were significantly increased in both sputum positive (2651 mg/dl, significant at 0.1% level) and sputum negative (2653 mg/dl, significant at 0.1% level) patients, though no significant deviation was observed between these two groups. The level of IgG was positively correlated with bilaterally extensive disease. The drug failure patients had higher activity of IgG than drug responders. A statistically significant increase of IgG level (2946.6 mg/dl) was observed in HLA-DR2 positive patients as compared to the DR2 negative groups (1678 mg/dl, significant at 0.1% level). No significant deviation was observed in the levels of IgM between any groups of the patients.

T cells, their sub-population, B cells and blasts those are responsible for the immune reaction of the disease were quantitatively assessed. The total lymphocytopenia was observed in the diseased patients of both pulmonary tuberculosis (2.0%) and pleural effusive (1.3%) as compared to controls (3.2%). Lower number of T cells was noted in peripheral blood mononucleocytes (PBMC) of PTB patients than in control. In pleural effusive patients the number was still lower in pleural fluid (PFL = 53%) and PBMC (51%). T cells revealed a significant decrease in bilaterally extensive disease. There was no correlation

between T cell activity and sputum positivity. Further T cells were found to be lower (57%) in drug failure patients. The percentage of CD4 cells was least in PBMC of PTB (33%) and lower in PBMC (44%) and PFL (41.1%) of pleural effusive patients as compared to healthy control (45.05%). There was no significant deviation in CD8 cells as compared to control except in pleural fluid where slightly higher percentage was observed. The ratio of CD4/CD8 was almost same in these cases. Further T cells were found to be lower (57%) in drug failure patients. The percentage of CD4 cells were least in PBMC of PTB (33%) and lower in PBMC (44%) and PFL (41.1%) of pleural effusive patients as compared to healthy control (45.05%). There was no significant deviation in CD8 cells as compared to control except a little higher in pleural fluid. The ratio of CD4/CD8 was almost same. The percentage of B cells was higher in all the patients group than normal and was highest in pleural fluid though no significant deviation was observed among the patient group.

Immunogenetic studies of the mycobacterial infectious diseases are important since the causative organism is already known. The importance of the study of association between PTB and HLA was the variability of the alleles associated with the ethnic population. HLA association with tuberculosis in geographically varied population may provide crucial information on the involvement of immunopathogenetic mechanisms.

In the present study a strong association of HLA DR2 with PTB patients was observed. In this regard it is need to be mentioned that this association was very strange among the drug failure patients. In addition with Chinese (Mehra *et al*, 1986), Indonesians (Bothamley *et al*, 1989), Kazakh, Russians (Khomenko *et al*, 1990) and in North (Singh *et al*, 1983) and South Indians (Brahmajothi *et al*, 1991) the present study also confirms the DR2 association with PTB patients in our population as well. So one can postulate that specific epitopes lies on the DR2 molecule may preferentially bind pathogenic mycobacterial peptides leading to the stimulation of CD4 T cell clones resulting the detrimental immune response for causing the disease clinically. Further molecular subtyping is needed to be done to find out the particular sequence of DR2 molecule where the epitope

exactly lies. This may provide further information for the identification of critical amino acid residues involved in peptide binding. Deleting such disease inducing epitopes and combining multiple immunity inducing peptides that can bind to all or most of the DR molecules will be the most useful in developing an effective/ protective antimycobacterial vaccine at the population level. In addition to HLA molecules the search for other disease susceptibility genes that are very closely linked to the HLA complex may yield useful information.

Quantitation of antibody levels can prove to be useful indicators of disease activity in term of active/detrimental cell mediated immunity against the invading pathogen. Specific epitopes derived from *M. tuberculosis* antigens/antibodies should be used to study the exact specificities of these antibodies. This will provide an important insight into the host anti-idiotypic network during anti-mycobacterial immunity.