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Major histocompatibility complex restriction in tuberculosis susceptibility

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Abstract. More than one mechanism may contribute to disease susceptibility in tuberculosis, viz., major histocompatability complex (MHC) restriction phenomenon, spectrum of immune reactivity/cytokine profile and epidemiology induced anergy. Experiments from our laboratories revealed that (i) human leucocyte antigen D-related allele 2 (HLA DR2) predispose for a more severe form of pulmonary tuberculosis encoding a high responder status, (ii) spectrum of immune reactivity to mycobacteria is 'innate', and it is demonstrable in healthy individuals from endemic area, (iii) there is no correlation between the purified protein derivative (PPD) response and peptide responses, (iv) once a person is high responder to P16 and P38 derived peptides (6/22), he/she (whether a patient or control) is a high responder for a wide range of mycobacterial peptides and (v)majority of the T-cell clones generated *in vitro*, to peptide 16·3 (amino acids 21-40) of 16 kA a mycobacterial antigen, in an HLA DR2 positive healthy individual is HLA DR restricted, permissive and of Th1 phenotype. The results suggested that MHC class II restriction play a role in peptide recognition and the immune response. Nonetheless the outcome and specificity of the immune reactivity and the resultant disease pathogenesis may depend on the promiscuity of peptide recognition and cytokine profiles.

Keywords. MHC restriction; mycobacterial diseases; immune reactivity; responder status; peptides; cytokines.

1. Introduction

The mechanism of association of HLA with diseases is still an enigma: this is more so with mycobacterioses, other chronic infections and parasitic diseases. In recent times, there is evidence for a stronger association of HLA DR2 with pulmonary tuberculosis (PTB) (Bothamley *et al* 1989; Khomenko *et* a/c 1990; Brahmajothi *et* al 1991). Many earlier studies showed associations with different HLA alleles in both class I and class II loci. One study on northern Indian population showed a nominal association with HLA DR2 (cf. Pitchappan 1990). Genome scan analysis of affected sib-pairs of diabetes using microsatellite markers revealed that, HLA alleles contribute to 54% of disease pathogenesis and the rest is accounted by three other genes (Davies *et al* 1994). A similar kind of approach is warranted in mycobacterioses. Nonetheless one need to understand the immunological mechanisms that predispose for mycobacterioses for a better therapy and prevention.

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There are many studies that evaluated both the HLA and immunological mechanisms in tuberculosis and leprosy (Nath *et al* 1980; Brahmajothi 1990; Rani *et al* 1992; Mehra *et al* 1995). Our study showed a strong HLA DR2 association with far advanced PTB (Brahmajothi *et al* 1991). Further, the existence of a spectrum of immune reactivity in healthy individuals has also been described by us for the first time (Pitchappan *et al* 1991). This has now been confirmed in Indonesian and Brazilian populations (Bothamley *et al* 1992; Fonseca *et al* 1992). However, the HLA association and spectrum of reactivity may be two independent mechanisms. In this paper we present our observations on these aspects.

2. Materials and methods

Patients attending Govt. Rajaji Hospital, were enrolled, informed consent obtained and studied. Controls were drawn from hospital staff, students and staff of our

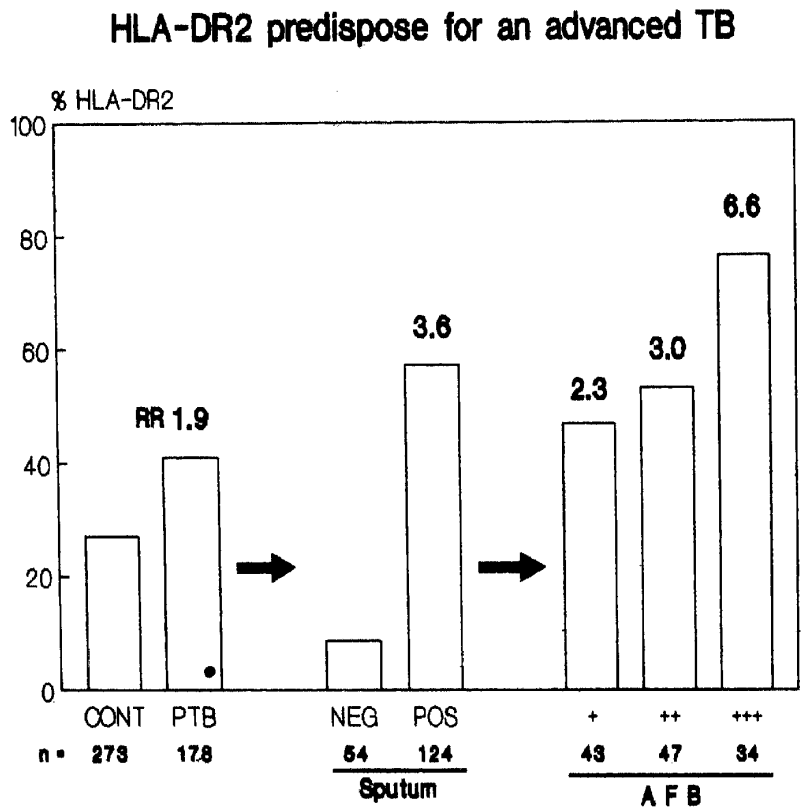

Figure 1. Association of HLA DR2 with pulmonary tuberculosis. One hundred and seventy eight patients (PTB) and 273 controls (CTL) were studied for their HLA and sputum acid fast bacilli (AFB) and % HLA frequency calculated. HLA DR2 showed a significant relative risk. Patients were classified based on the presence of AFB in their sputum smear and culture (SP N sputum negative; SP P, sputum positive) and % DR2 calculated. The sputum positive patients were further stratified, based on the number of AFB in their sputum smear, bacterial count in culture and based on the radiological lesions. Three categories were identified viz. + (mild), + + (moderate) and + + + (far advanced) and DR2 frequency calculated and presented in the histogram. The results were similar in all the three methods of classification. The number above each bar represents the relative risks in the respective group: note that the relative risk was the highest in the + + + AFB category.

university. Serological typing of HLA was done by conventional two stage microlymphocytotoxicity assay (Terasaki and McClelland 1964). Allelic typing of HLA was done by (PCR-SSP) polymerase chain reaction-sequence specific primers and sequence specific oligo probes (SSOP) techniques (Olerup and Zetterquist 1992). Lymphocyte transformation test (LTT) assays and T-cell cloning was done following standard procedures (Mosmann *et al* 1986; Street *et al* 1990). Monoclonal antibodies employed were commercial ones and peptides were synthesized based on the known sequences of 16 and 38 kDa antigens of *Mycobacterium tuberculosis.* Standard statistical methods were employed to analyse the data (Snedecor and Cochran 1968), employing the software developed for immunogenetic studies (Pitchappan and Arulraj 1989).

3. Results and discussion

3.1 *HLA DR2 association*

Figure 1 presents the data on the distribution of HLA DR2, a high risk allele, in various subgroups of patients with pulmonary tuberculosis. While % frequency of HLA DR2 was 27 in controls, it was 41 in PTB patients. On further stratification of patient samples, in sputum positive it was 57%, and in far advanced among them, it was 76. This suggested that if a person is infected and if that individual posses HLA DR2, his/her chances of developing a more severe, sputum positive (+ + +), culture positive (+ + +) and radiologically far-advanced PTB is greater than other alleles (cf. Brahmajothi 1990; Brahmajothi *et al* 1991). Further patients with HLA DR2 showed higher antibody levels

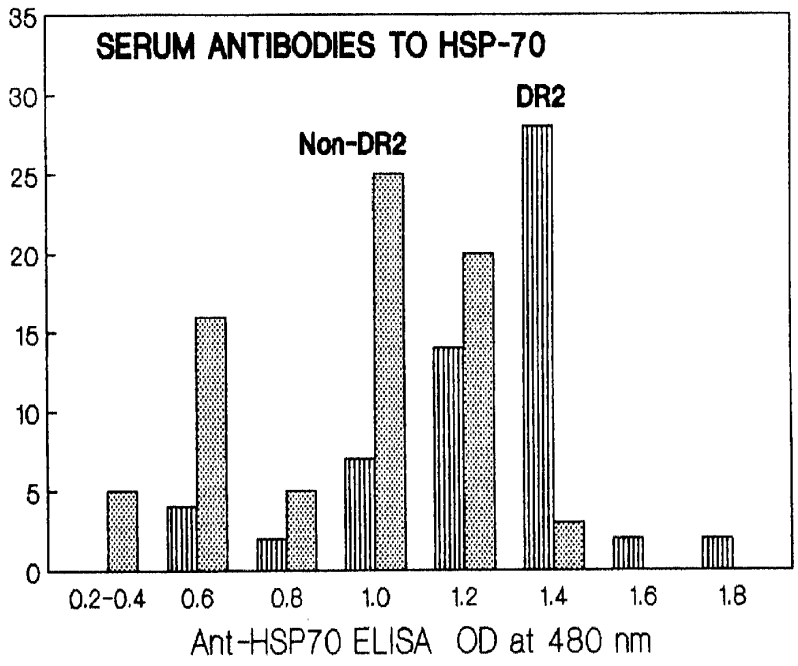

Figure 2. HLA DR2 and serum antibodies. Anti-HSP-70 (IgG) antibodies were estimated by ELISA in DR2 and non-DR2 patients. The results are presented as 0·2 OD class interval starting from 0·0–0·2. The Y axis represents the number of patients in each OD class.Note that more number of DR2 patients showed higher OD classes.

Table 1. HLA DR2 association with pulmonary tuberculosis in different populations.

Place	Year	Pts/Ctl	%PF	RR	X2	EF	DR2 associated with SP+	SP−	HR	EB
Indonesia	1989[a]	101/64	56/31	2·8	9·4	0·36	Y	–	Y	–
U S S R	1990[b]	643/984	–	–	–	–	Y	–	Y	Y
South India	1991[c]	143/287	52/33	2·2	15	0·29	Y	N	Y	Y

Cf. Pitchappan (1990) for a compilation of the earlier works on HLA association and showed a significant DR2 association.
[a]Boathamley *et al* 1989, [b]Khomenko *et al* 1990, 'Brahmajothi *et al* 1991.
Pts, patients; Ctl, controls; % PF phenotype frequency x 100; RR, relative risk; EF, etiological fraction; SP +, sputum positive; SP — , sputum negative; HR, high responders; EB, association transcend ethnic barriers. N, No; Y, yes.

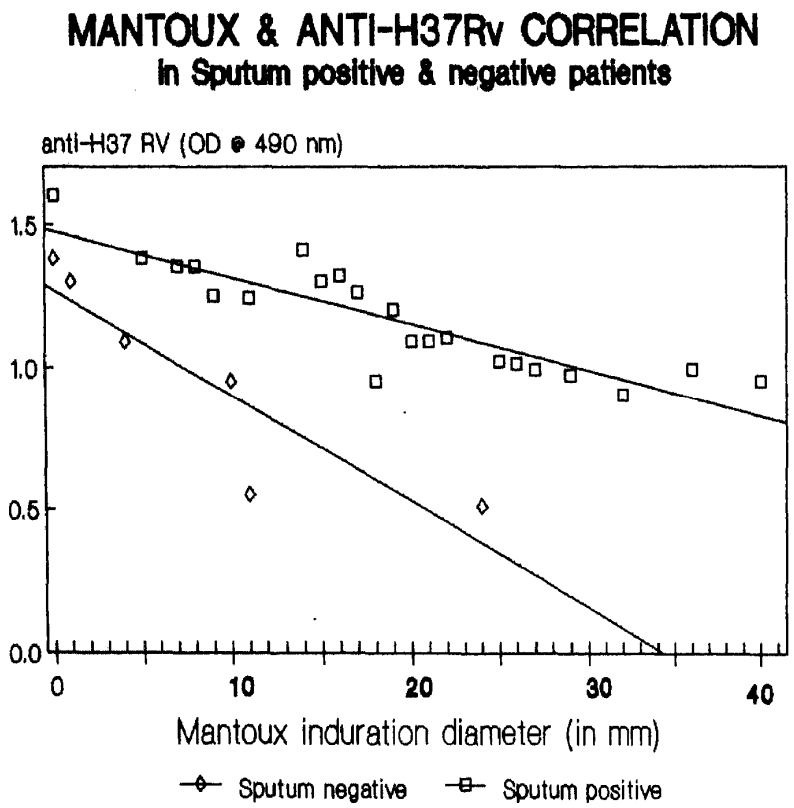

Figure 3. Spectrum of immune reactivity in sputum positive and sputum negative patients. Anti-H37Rv antibodies of IgG isotype of sputum positive and sputum negative patients were plotted against their respective Mantoux induration diameter. Note the differences in the slope of these two regression lines.

than non-DR2 particularly in anti-HSP7O (figure 2) and in anti-PPD (Brahmajothi *et al* 1991). There are two other studies confirming the HLA DR2 association (table 1): all these studies suggested that HLA DR2 correlates to a high responder status irrespective of mycobacterial antigen preparation used. Further, our study and Russian study confirmed that the HLA DR2 association transcend ethnic barriers (table 1).

3.2 *Spectrum of immune reactivity*

We provide evidence that the spectrum of immune reactivity [two types of immunity viz., cell mediated (CMI) and humoral (HI)], demonstrable in patients with leprosy and tuberculosis, exists in healthy hospital contacts (Pitchappan *et al* 1991). This has now been confirmed and extended to healthy populations in Indonesia and Brazil (Bothamley *et al* 1992; Fonseca *et al* 1992). This inverse correlation (of CMI and HI) was observed in both sputum positive and negative patients as well. Nonetheless there was a quantitative and qualitative difference between these two patient groups (figure 3). This spectrum of immune reactivity correlated to the recovery of patients by chemotherapy and to the drug resistant statuses. The early converters possessed a good CMI but low antibody levels and the patients with drug resistant bacilli possessed a poor CMI and an elevated antibody level (Horton 1995; V Brahmajothi, R M Pitchappan, C N Paramasivan, K Rajaram, U Sankarkumar and R Prabhakar, unpublished results). In other words patients with a good CMI have a better prognosis. One may need to examine whether the good HI in poor prognostic groups are related to antibodies against drug resistant organisms: our study however has not answered this question directly. Nonetheless it is possible that the drug resistant organisms induce a higher levels of antibody leading to a more severe damage and persistence and therefore the delay in recovery by chemotherapy.

In recent times immunity against mycobacterial infections is thought to depend on HLA class II restricted Thl cells (Ottenhoff 1996). Both type one (Thl cells) and type two (Th2 cells) cytokines have been reported in many tuberculosis and leprosy patients (Barnes *et al* 199O; Misra *et al* 1995). Cytokine profile of PBL and cells in the lesions of leprosy patients from India has revealed the presence of both indiscriminate (Th0) and polarized (Th1 or Th2) T-cell subsets, in both tuberculoid leprosy (TL) and lepromatous leprosy (LL) patients. Further, no difference in the pattern of cytokine secretions between peripheral blood lymphocytes (PBL) and lesional lymphocytes and various mycobacterial antigens have been identified (Misra *et al* 1995). Though the skewing of T-cell subsets and their cytokine profile may be an important mechanism in the pathology of mycobacterial diseases, their role in susceptibility to the disease needs to be evaluated. It is possible that the spectrum of immune reactivity observed in healthy individual from endemic areas is the outcome of their cytokine profile (i.e., their Th1/Th2 status to the antigen in question) and this might decide the course of the resulting susceptibility, immunity and pathology. The proposition raises another interesting question whether the Th 1 or Th2 response to a given antigen/epitope is inherent (innate), and whether it can be skewed in an endemic area.

3.3 *High and low responder status to peptides*

In order to study the role of MHC restriction in disease pathogenesis, lymphocyte transformation test (LTT) responses of patients and controls to selected mycobacterial peptides were studied and correlated to their MHC class II (HLA DRB1, DRB5, DQA and DQB) haplotypes, assigned by PCR-SSP typing. There was no correlation between any given haplotype and allele to either high or low response to any particular peptide studied. Rather a high responder to a peptide irrespective of DR2 status, always responded high to many peptides, studied (figure 4) but not to PPD. To a descending

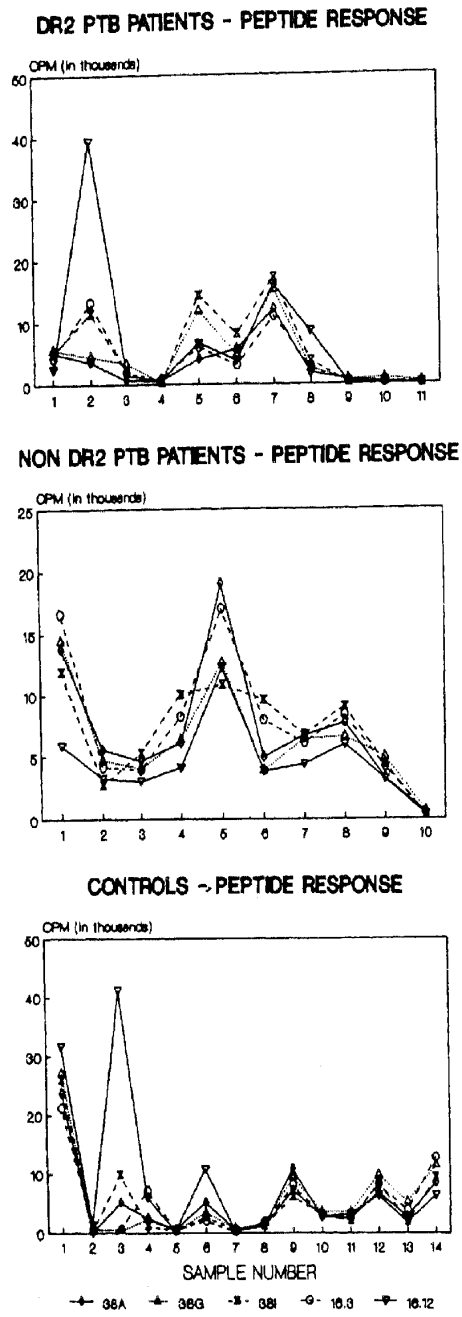

Figure 4. LT response of DR2 and non-DR2 PTB patients and controls to various mycobacterial peptides. LTT responses were studied in microplates following conventional methods and tritiated thymidine incorporation assays. Mycobacterial peptides (M. *tub)* 38A, 38G, 381, 16·3, 16·12 and PPD were studied in DR2 positive patients (N = 11), non-DR2 patients (11) and controls (14). Each data is the average of triplicate. The X-axis represents various individuals and they were arranged in the descending order of the PPD response (refer figure 5) and Y-axis counts per min. Note that if an individual responds to a given peptide, he/she responds to the same extent for other peptides studies as well: nonetheless this did not correlate to the PPD response.

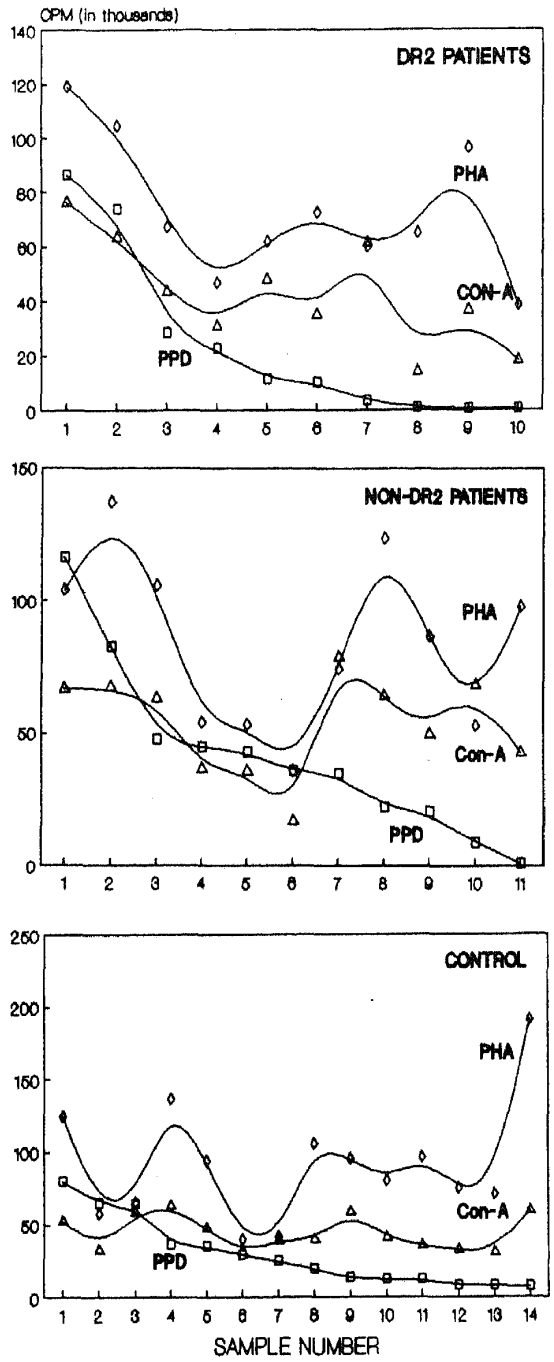

Figure 5. Comparison of PPD and PHA/Con A responses in DR2 and non-DR2 patients. Ten numbers of DR2 patients, 11 non-DR2 patients and 14 controls were studied for their proliferative responses to PPD, PHA and Con A (each data is the average of triplicate). The data on these samples were arranged on a descending order of PPD response and their PHA and ConA responses plotted. Note that PHA and Con A responses were higher at both the extremes of PPD responses compared to median responders. Thus the PPD responses did not correlate to PHA and Con A responses.

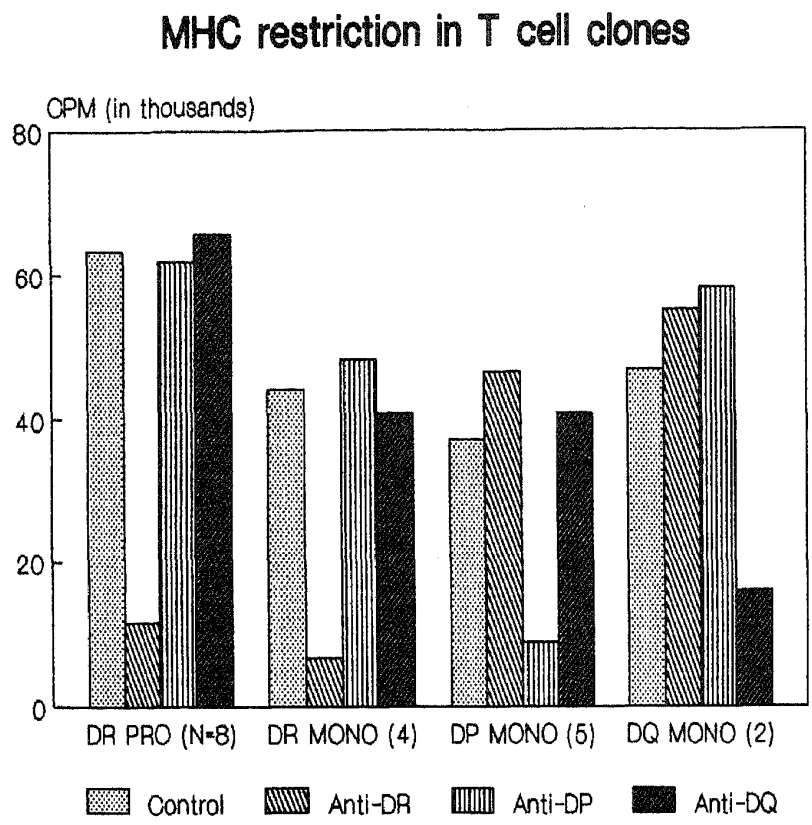

Figure 6. MHC restriction of T-cell clones generated from an HLA-DR15/DR9 heterozygote healthy individual. Nineteen Th clones were generated in response to peptide 16·3 in bulk culture and limiting dilution analysis. These clones were tested for their peptide 16·3 dependant proliferation in LTT assay, in the presence of monoclonal antibodies to HLA-DR, HLA-DQ or HLA-DP. Proliferations of 12 clones were suppressed by anti-DR monoclonal antibodies, five by anti-DP and two by anti-DQ thus identifying their MHC class II restriction status. Of the 12 DR restricted clones eight were promiscuous and four, monogamous (see figure 7).

order of LTT response to PPD, photohaemagglutinin (PHA) and Concanavalin-A (ConA) curves was bimodal, both in patients and controls (figure 5). The two polar groups of LTT response with higher PHA stimulation might be qualitatively different from one another and compared to the median responders.

3.4 *T-cell cloning studies*

Nineteen T-cell clones were generated against peptide 16·3, in a normal HLA DR15/DR9 heterozygous healthy donor. Of these clones 63% was HLA DR restric ted. Majority of these DR restricted clones (72·7%) were promiscuous in nature, i.e., they recognized the peptides presented by antigen presenting cells (APC) of either HLADR15 or DR9, the parental alleles (figure 6) (N Javed, Sara Deacock, R Wilkinson, R M Pitchappan and Ivanyi, unpublished results). Twenty-six percent of the clones generated were restricted by HLA DP4 (parental HLA DP) and 10% by DQ2 (parental HLA DQ) and all of them were monogamous. The MHC restriction was

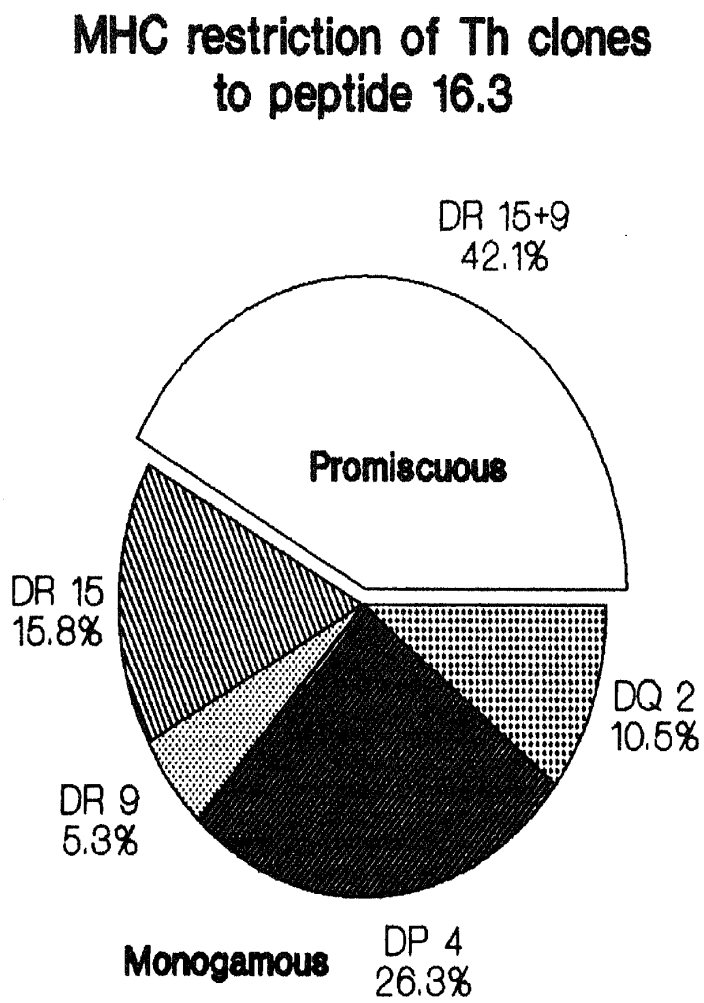

Figure 7. Promiscuity in MHC restriction. T-cell clones were presented with the peptide 16·3 by various antigen presenting cells [homozygous epstein bar virus (EB V) cell lines with different HLA DR]. The antigen presenting cells (APCs) were pulsed with the peptide, presented to the clones and proliferation of the clones studied in a tritiated thymidine incorporation assay. The clones responded only when the peptide was presented by the respective restricting elements, i.e., one parental MHC (HLA DR15, DR9, D Q2 or DP4). If the clone was HLA DR15 restricted, it proliferated only when it was presented with the peptide by an APC carrying HLA DR15. However 72·7% of the DR restricted clones (42% of the total clones) recognized the peptide and proliferated when the peptides were presented by APCs carrying either HLA DR 15 or HLA DR9, indicating the promiscuity of these clones. Note that these clones did not know to recognize the peptides when presented by APCs carrying other than these two parental HLA DR alleles. All the DP and DQ restricted clones were monogamous, i.e., recognized the peptide in the context of any one of the parental alleles.

evaluated by blocking the LTT response with HLA DR, DQ and DP locus specific monoclonal antibodies (figure 7). The study further revealed that majority (75%) of DR restricted, promiscuous clone were of Thl phenotype. The study revealed that (i) in response to a single peptide 16·3 *in vitro*, many clones can be generated (recall/memory response), (ii) each clone is restricted by one or the other MHC class II alleles of the host,

(iii) the HLA DR restricted clones were the predominant one, presumably easily inducible by the mycobacterial antigen in question and (iv) many HLA DR restricted clones were promiscuous in nature. Thus the disease may be the outcome of the interactions of all these clones *in vivo,* and the genetics of the host may play a predominant role in deciding whether the disease will manifest and progress in an infected (sub-clinically) host.

4. Conclusion

The mechanism of association of HLA DR2 and the spectrum of immune reactivity in tuberculosis might be two parallel but independent mechanisms. During an immune response *(in vitro),* clones restricted by various MHC loci with different cytokine profile can be generated. The existence of high or low responder status to many mycobacterial peptides but not to PPD suggests that MHC diversity, mycobacterial peptides generated in a given *milieu genetic,* T-cell receptor (TCR) repertoire, epidemiology and cytokine profile, all may decide the disease susceptibility. Identifying the combinations of these factors leading to disease/protection is a challenging problem to immunologists.

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References

Barnes P F, Fong S J, Brennan P J, Twomey P E, Mazumder A and Modlin R L 1990 Local production of tumor necrosis factor and interferon gamma in tuberculosis pleuritis; *J. Immunol.* **145** 149-154

Bothamley G H, Beck J S, Schreuder G M Th, D'Amaro L S, de Vries R R P, Kardfjito T and Ivanyi J 1989 Association of Tuberculosis and *Mycobacterium tuberculosis-Specific* Antibody levels with HLA; *J. Infect. Dis.* **59** 549-555

Bothamley G H, Rudd R, Festenstein F and Ivanyi *J* 1992 Clinical value of the measurement of *M. tuberculosis-specific* antibody in pulmonary tuberculosis; *Thorax* **47** 270-275

Brahmajothi V 1990 *Studies on the HLA association in Health and diseases among selected groups of Tamil Nadu,* Ph.D thesis, Madurai Kamaraj University, Madurai

Brahmajothi V, Pitchappan RM, Kakkanaiah V N, Sasidhar M, Rajaram K, Ramu S, Palanimurugan K, Paramasivam C N and Prabhakar R 1991 Association of Pulmonary tuberculosis and HLA in South India; *Tubercle* **72** 123-132

Davies J L, Kawaguchi Y, Bennet S T, Copeman J B, Cordall H J, Pritchard L E, Reed P W, Gough S C L, Jenkins S C, Palmer S M, Balfour K M, Rowe B R, Farrall M, Barnett A H, Bain S C and Todd J A 1994 A genome-wide search for Human type 1 diabetes succeptibility genes; *Nature* (*London*) **371** 130-136

Fonseca L S, Saad M H F and Martins F M 1992 The spectrum of immune response to M. *tuberculosis* in healthy individuals; *Tubercle Lung Dis* **73** 242

Horton R 1995 Towards the elimination of tuberculosis; *Lancet* **346** 790

Khomenko A G, Litvinov V Chukanova V P and Pospelov L E 1990 Tuberculosis in patients with various HLA phenotypes; *Tubercle* **71** 187-192

Mehra N K, Rajalingam R, Mitra D K, Taneja V and Giphart M J 1995 Variants of HLA-DR2/DR51 group haplotypes and susceptibility to tuberculoid leprosy and pulmonaray tuberculosis in Asian Indians; *Int. J. Lepr. Other Mycobact. Dis.* **63** 241-248

Misra N, Murtaza A, Walker B, Narayan N P, Misra R S, Ramesh V, Singh S, Colston M I and Nath 1 1995 Cytokine profile of circulating T cells of leprosy patients reflects both indiscriminate and polarized T-helper subsets: T helper phenotype is stable and uninfluenced by related antigens of M. *leprae; Immunology* **86** 97-103

Mosmann T R, Cherwinski H, Bond M W, Giedlin M A and Coffman R L 1986 Two types of murine helper T cell clones. I. Definition according to profiles of lymphokine activities and secreted proteins; *J. Immunol.* **136** 2348-2352

Nath I, vanRood J J, Mehra N K and Vaidya M C 1980 Natural suppressor cells in human leprosy: the role of HLA-D identical peripheral lymphocytes and macrophages in the in vitro Modulation of lympho-proliferative responses; *Clin. Exp. Immunol.* **42** 203-208

Olerup 0 and Zetterquist H 1992 HLA-DR typing by PCR amplification with sequence specific primers (SSP-PCR) in two hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation; *Tissue Antigens* **39** 225-232

Ottenhoff T H M 1996 Specificity, function and regulation of functionally different T cell subsets in mycobacterial immunity; in *Third Int. Conf on the pathogenesis of mycobacterial infections*, Stockholm, Sweden. Abstracts pp 16

Pitchappan R M 1990 Genetics of tuberculosis susceptibility; *Trop. Med. Parasitol.* **41** 355-356

Pitchappan R M and Arulraj N 1989 A versatile computer Data-base for Human Immunogenetic Studies; *Indian J. Physiol. Anthropol. Human Genet.* **15** 81-92

Pitchappan R M, Brahmajothi V, Rajaram K, Subramanyam P T, Balakrishnan K and Muthuveeralakshmi R 1991 Spectrum of Immune reactivity to mycobacterial (BCG) antigens in healthy hospital contacts in South India: *Tubercle* **72** 133-139

Rani R, Zaheer S A and Mukherjee R 1992 Do human leukocyte antigens have a role to play in differential manifestations of multibacillary leprosy: A study on multibacillary leprosy patients from North India; *Tissue Antigens* **40** 124-127

Snedecor G W and Cochran W G 1968 Statistical methods (Iowa: State University Press)

Street N E, Schumacher J H, Fong T A T, Bass H, Fiorentino D F, Leverah J A and Mosmann T R 1990 Heterogeneity of mouse helper T cells: evidence from bulk cultures and limiting dilution cloning for precursors of Thl and Th2 cells; *J. Immunol.* **144** 1629-1636

Terasaki P I and McCleeland J D 1964 Microdroplet assay of human serum cytotoxins; *Nature* (*London*) **204** 998-1001