Influence of HLA-DR2 phenotype on humoral immunity & lymphocyte response to *Mycobacterium tuberculosis* culture filtrate antigens in pulmonary tuberculosis

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Association of HLA-DR2 genes/gene products has been shown with pulmonary tuberculosis (PTB) patients in India. In the present study, the influence of HLA-DR2 and non-DR2 genes/gene products on immunity to tuberculosis has been studied. Plasma samples of -DR2 positive patients (active and inactive TB) showed a higher antibody titre to *Mycobacterium tuberculosis* culture filtrate antigens than non-DR2 (-DR2 negative) patients. Immunoblot analysis revealed a trend towards an increased percentage of DR2 positive patients recognizing 38, 32/34 and 30/31 kDa antigens of *M. tuberculosis* than DR2 negative patients. A low spontaneous lymphoproliferative response (without antigen stimulation) was seen in HLA-DR2 positive active TB patients than HLA-DR2 negative patients. However, the antigen stimulated lymphocyte response was higher in the -DR2 positive patients (active and inactive TB) when compared to non-DR2 patients. Further, an inversional correlation between antibody titre and spontaneous as well as antigen induced lymphocyte response (measured by ^{3}H thymidine uptake and expressed as counts per minute) was seen in HLA-DR2 positive active PTB patients than non-DR2 patients. The present study suggests that HLA-DR2 genes/gene products may be associated with a regulatory role in the mechanism of disease susceptibility to tuberculosis. The genes while augmenting the humoral immune response, they suppress the spontaneous and antigen induced lymphocyte response in -DR2 positive patients with active disease.

Key words Antigen recognition - HLA-DR2-antibody titre - lymphocyte response - *Mycobacterium tuberculosis* culture filtrate antigens - pulmonary tuberculosis patients

Human leucocyte antigens (HLA) especially the class-II antigens have been shown to influence immune functions. The products of the HLA genes mediate the recognition and immune response to any antigen or pathogen by presenting antigens to T lymphocytes[1]. The secretion of cytokines such as tumour necrosis factor- α and interleukin- 1 [2] as well as the lymphocyte response to antigens[3,4] are influenced by HLA genes and their gene products.

Several studies have been carried out to understand whether the susceptibility and/or immune response to *Mycobacterium tuberculosis* is associated with HLA phenotype and / or controlled by genes linked to the major histocompatibility complex[3,5-12]. Studies in the Asian region, especially in India[6-10], Indonesia[5,11] and Russia[12] showed a significant increase of HLA-DR2 antigen frequency in pulmonary tuberculosis patients. Our recent study on the role of HLA-class II genes/gene products on lysosomal enzymes revealed that HLA-DR2 phenotype is associated with a decreased plasma lysozyme level (an enzyme involved in the non-

specific killing mechanism of micro organisms) in active pulmonary tuberculosis (PTB) patients[13]. It has been shown in Russians that PTB patients positive for HLA-DR2 had higher levels of antibodies to *M. tuberculosis* than other patients[12]. Further, humoral immune response to few epitopes of 38 kDa antigen of *M. tuberculosis* is restricted to HLA-DR2[5,11].

The influence of HLA-DR2 on humoral and cell mediated immunity in active TB patients, cured patients and normal healthy subjects has not been fully established. The present study was aimed at understanding the role of HLA-DR2 genes/gene products on antibody and lymphocyte response to *M. tuberculosis* culture filtrate antigens in pulmonary tuberculosis patients and control subjects. The influence of HLA-DR2 on immunity to tuberculosis has been studied in both active and inactive stages (cured) of the disease. Moreover, the influence of *M. tuberculosis* antibodies on lymphocyte response in HLA-DR2 positive and -DR2 negative patients and controls has also been studied.

Material & Methods

Study subjects

(i) Active tuberculosis patients (ATB) – Patients attending the Tuberculosis Research Centre (TRC), Chennai, with respiratory symptoms and radiographic abnormalities suggestive of pulmonary TB were studied. These patients had sputum positive for *M. tuberculosis* by smear and culture. Blood samples were taken before the start of chemotherapy. Among 55 ATB patients studied, 48 were males and 7 were females. The mean age with SE was 38.3±1.7 for males and 29.3±3.7 for females.

(ii) Inactive tuberculosis patients (ITB; cured patients) – Patients classified as suffering from active pulmonary tuberculosis were given anti-tuberculosis treatment at our Centre. All these patients had received short course chemotherapy for 6-8 months. These patients had been treated 10-15 yr earlier. At the time of blood sample collection all these cured patients were in the quiescent stage of the disease. Of the 89 cured patients studied, 66 were males and 23 were females. The mean age with SE was 38.9±1.3 for males and 35.0±1.9 for females.

(iii) Controls – Spouses of cured TB patients living together for 10-15 yr (family contacts; n=25) and clinicians, social workers, health visitors, laboratory volunteers and other staff (n=37) working at TRC for more than 3 years were studied. Among the 62 control subjects, 29 were males and 33 were females. The mean age with SE was 40.1±1.6 for males and 37.6±1.6 for females. All the family contacts and other control subjects were clinically normal at the time of blood sample collection.

The patients and spouses were not consanguinous to each other. The other control subjects were not related to any of the patients. The patients and the controls were randomly selected and belonged to the same ethnic group. They were Tamil speaking south Indian population (belonging to different communities) living in and around Chennai.

HLA typing : Peripheral blood mononuclear cells (PBMC) were separated from heparinised blood samples using ficoll-hypaque density gradient centrifugation as described by Boyum[14]. T- and B-lymphocytes were separated from PBMC using nylon wool column[15] and adherent cell population (enriched B lymphocytes) was used for HLA-DR typing using two stage microlymphocytotoxicity assay[16]. Commercial sources of antisera (Biotest, Frankfurt, Germany) were used for HLA-DR typing and at least 3 antisera were used for each specificity.

M. tuberculosis culture filtrate antigen : $H_{37}Rv$ strain of *M. tuberculosis* was grown for 6 wk (late logarithimic phase) as a surface pellicle on Sauton's medium at 37°C. The culture was centrifuged at 3,000 rpm for 30 min to remove the bacilli and the supernatant was filtered through a Seitz filter (0.45 μm; Seitz-Filter-Werke GmbH und Co., Badkreuznack, Germany) to ensure complete removal of the bacilli. The culture filtrate antigens were precipitated with 80 per cent ammonium sulphate and dissolved in sterile phosphate buffered saline (PBS; pH 7.4). The excess salt was removed by dialysis using 0.2M, 0.1M and 0.02M PBS (pH 7.4) at 4°C. The dialysed protein was lyophilised and resuspended in minimal volume of sterile PBS. The protein concentration was estimated by Lowry's procedure[17], against bovine serum albumin (BSA) as

standard. The culture filtrate protein antigens were aliquoted and stored at -20°C until required.

Enzyme linked immunosorbent assay (ELISA) : The IgG antibody titre against *M. tuberculosis* culture filtrate antigens was measured by ELISA. The ELISA plates (polyvinyl chloride; Costar, Cambridge, MA, USA) were coated with 5 µg/ml concentration of *M. tuberculosis* $H_{37}Rv$ culture filtrate antigens. Plasma samples were serially diluted in PBS (from 1:5 to 1:20480) in the plate using a multichannel pipette. The plates were incubated at 37°C for 1 h. Rabbit anti-human IgG peroxidase conjugate (Genei, Bangalore Genei Pvt. Ltd., Bangalore) was used as the second antibody in 1: 1000 dilution and incubated at 37°C for 1 h and washed in PBST (IM PBS pH 7.4 and 0.1% Tween 20). The colour was developed using orthophenylenediamine (OPD 0.5 mg/ml) (Sigma Chemical Co., St. Louis , USA) and 0.1 per cent hydrogen peroxide as substrate. The optical density was measured at 490 nm in an ELISA reader (Titertek multiscan plus, MKII, Flow Laboratories International, Lugano, Switzerland). Twice the mean optical density (OD) of the blank value was taken as the cut-off point. Plasma samples showing OD values above the cut-off were taken as positives and the corresponding titre values were recorded.

Immunoblotting : M. tuberculosis ($H_{37}Rv$) culture filtrate antigen (50 µg/cm gel) was loaded onto a 12.5 per cent sodium dodecyl sulphate polyacrylamide slab gel electrophoresis (Protean, Bio-Rad Laboratories, California, USA) using a discontinuous tris-buffered system as described elsewhere[18]. A molecular weight marker was run along with the antigen and stained with 0.5 per cent Coomasie brilliant blue (R250) (Bio-Rad Laboratories, California, USA). After electrophoresis, proteins were transferred electrophoretically to nitrocellulose, paper (NCP) (Advanced Microdevices Pvt. Ltd., Ambala) by the method of Towbin *et al* [19], in a blot apparatus (Transblot apparatus, Bio-Rad Laboratories, California, USA) for 1 h at 65 mA constant current. The NCP strips were treated with the plasma samples of the patients (1:50 dilution), rabbit anti-human IgG peroxidase conjugate as second antibody (1: 1000 dilution), (Genei, Bangalore Genei, Bangalore), hydrogen peroxide as substrate and colour was

developed using diamino-benzidine (0.04%) (Bio-Rad Laboratories, California, USA) in PBS as the chromogenic substance. One strip for positive control (hyper immune rabbit serum against *M. tuberculosis* $H_{37}Rv$ strain) was included in each set of experiments. Anti-rabbit IgG peroxidase was used as second antibody (1:5000).

Lymphocyte transformation test : Peripheral blood mononuclear cells (PBMC) of ATB and ITB patients and controls were suspended in RPMI tissue culture medium (Sigma Chemical Co., St. Louis, USA) supplemented with 2mM L-glutamine ((Flow Laboratories, Irvine, Scotland, UK), gentamycin (10 µg/ml) (M.A. Bio Products, Walkerville, Maryland, USA), fungizone (5 µg/ml) (Squibb, Princeton, NJ, USA) and 10 per cent autologous plasma for controls and cured patients. Autologous serum was used instead of plasma in the cultures set up with active tuberculosis patients samples to avoid clotting. No difference in the counts were observed when autologous plasma or serum was used. 0.1×10^6 PBMC in 200 µl were cultured with or without 10 µg/ml concentration of *M. tuberculosis* culture filtrate antigens for 6 days in a 96 well 'U' bottom tissue culture plate (Costar, Cambridge, MA, USA). at 37°C and 5 per cent carbon dioxide in a CO, incubator (Flow Laboratories Ltd., Rickmansworth, Hertz, UK). Tritiated thymidine [(^{3}H) BARC, Trombay, Mumbai] was added to each well (1µCi/well) 16 h before termination. The cells were harvested onto glass fibre filters using a PHD cell harvester (Cambridge Technology, Inc, Water Town, MA, USA). The lymphocyte response to *M. tuberculosis* antigen was measured by tritium labelled thymidine uptake by the cells in a scintillation system and Beta counter (LKB, Wallac Oy, Turku, Finland) and expressed as counts per minute (cpm). The mean cpm value of the triplicate culture was calculated and the stimulation index (SI) was derived[20] using the formula:

$$\text{SI}: \frac{\text{mean cpm of antigen stimulated cell cultures}}{\text{mean cpm of cell cultures without antigen}}$$

Statistical analysis : The frequencies of HLA-DR antigens in patients and controls were determined by direct allelic count. The results on antibody titre and lymphocyte response are expressed as mean ± SE. Student's 't' test was used to see the significance of

the data. Significant *P* values were further corrected (Pc) by multiplying the *P* value for the number of antigens studied for -DR locus. Proportionality test was used for immunoblot analysis to find out the significance.

Results

A trend towards an increased antibody titre to *M. tuberculosis* culture filtrate antigen was seen in HLA-DR2 positive active and cured TB (inactive TB) patients than -DR2 negative patients. However, a significant increase in the antibody titre was seen only in cured TB patients (DR2 + ve vs DR2 - ve : *P* < 0.001 : Pc < 0.01) and not in the active TB patients (Fig. 1).

Most of the DR2 positive and DR2 negative ATB patients recognized 38 kDa antigen. However, an intense recognition pattern of 38 kDa antigen by the plasma of DR2 positive ATB patients was seen when compared to non-DR2 (-DR2 negative) ATB patients. Further, the plasma samples of the -DR2 positive cured (ITB) patients showed an increased recognition of 38 kDa antigens. However, this increase was not significant *(P* > 0.05). Moreover, a trend towards an increased recognition of 32/34 and 30/31 kDa *M.*

tuberculosis antigen complexes were seen in the -DR2 positive patients (ATB and ITB) and control subjects than -DR2 negative individuals. However, these increased trends were not significant (Fig. 2).

A low spontaneous lymphoproliferative response (without antigen stimulation) was seen in HLA-DR2 positive ATB patients (1,981.1 ± 279.3 cpm) than -DR2 negative patients (7,785.5 ± 2.979.5 cpm). However, the difference was not significant *(P* > 0.05). A similar trend was also seen in -DR2 positive cured (inactive TB) patients. However, such a trend was not evident in -DR2 positive control subjects (Table).
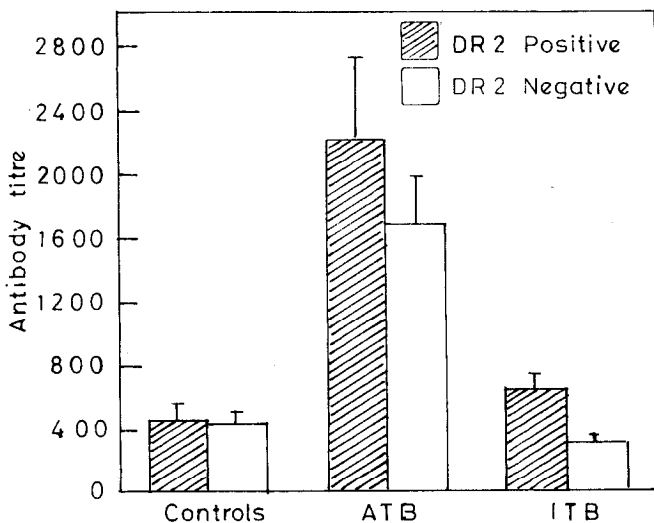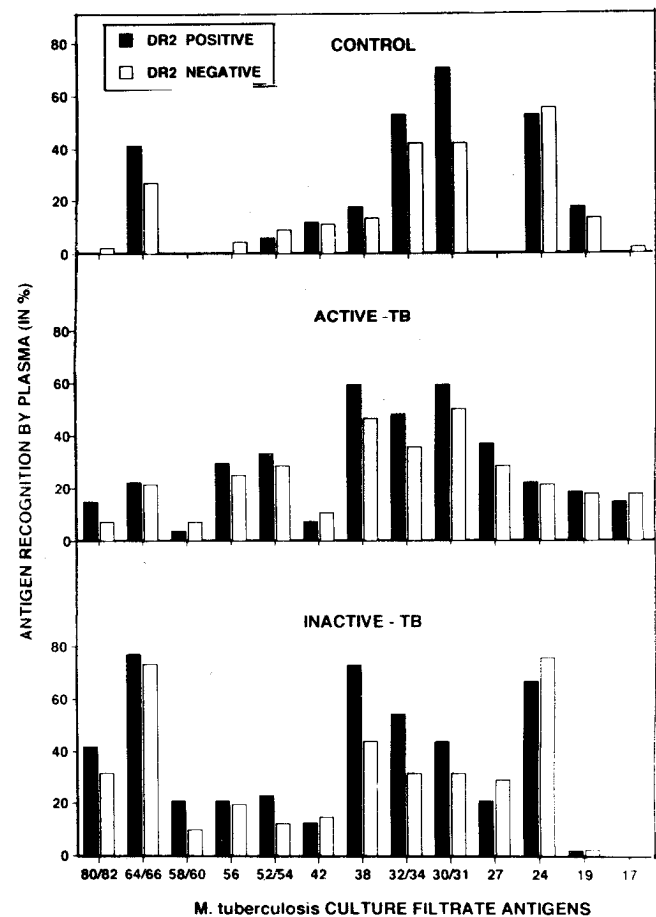

Fig. 1. HLA-DR2 and antibody response to *M. tuberculosis* culture filtrate antigen in control, active-TB and inactive-TB patients. Results are expressed as mean ± SE. ATB : DR2+ve vs DR2 -ve : *P* > 0.05; ITB : DR+ve vs DR2-ve : *P* < 0.001; Pc < 0.01; Pc-corrected for the number of DR antigens studied (i.e., DR1 to DR10).

Fig. 2. *M. tuberculosis* culture filtrate antigen (molecular weight in kDa) recognition pattern by plasma samples of HLA-DR2 positive and -DR2 negative control subjects and pulmonary tuberculosis (ATB and ITB) patients. Controls : DR2 +ve, n = 17 and DR2 -ve, n = 45; Active-TB : DR2 +ve, n = 27 and DR2 -ve, n = 28: and Inactive-TB : DR2 +ve, n = 48 and DR2 -ve, n = 41.

Table. Influence of HLA-DR2 on spontaneous and *M. tuberculosis* culture filtrate antigen induced lymphocyte response in control subjects and patients

| | | Counts per minute (cpm) | | |
	HLA DR2	Spontaneous response	*M. tuberculosis*[#] Antigen induced response	Stimulation index
Control subjects n=50	Positive n=13	3,523.4±1,252.4	22,309.9±5.085.4	6.3** (14.1±3.5)*
	Negative n=37	3,299.1±526.5	23,112.4±3,480.1	7.0** (12.2±2.2)*
Active-TB patients n=44	Positive n=20	1,981.1±279.3	10,486.4±2,525.0	5.3** (6.7±2.1)*[+]
	Negative n=24	7,785.5±2,979.5	14,206.8±4,461.3	1.8** (2.7±0.4)*[$]
Inactive TB patients n=38	Positive n=21	1,244.5±254.7	29,754.8±5,217.1	23.9** (34.5±9.2)*
	Negative n=17	2,286.8±923.0	29,610.5±5,462.4	13.0** (24.6±5)*

$P^{+} < 0.05$ as compared to DR2 +ve ITB patients

$ < 0.001$ as compared to DR2 -ve ITB patients

10 mg/ml concentration of *M. tuberculosis* culture filtrate antigen was used in the culture; ** Stimulation indices calculated collectively for all subjects; * Stimulation indices calculated for each subject separately and expressed as mean ± SE

The *M. tuberculosis* culture filtrate antigen induced lymphocyte response (Stimulation Index:SI) was higher in -DR2 positive ATB (6.7 ± 2.1) and ITB patients (34.5 ± 9.2) than -DR2 negative ATB (2.7 ± 0.4) and ITB patients (24.6 ± 5.0). However, the differences were not significant (P > 0.05). The -DR2 positive control subjects did not show an increased response when compared to -DR2 negative subjects. Further, the antigen induced lymphocyte response (SI) was lower in the active TB patients than inactive TB patients (DR2 +ve ATB vs DR2 +ve ITB : *P* < 0.05; non-DR2 ATB vs non -DR2 ITB : *P* < 0.001; Table).

The influence of antibody titre on spontaneous and antigen induced lymphocyte response and vice versa were analysed for -DR2 positive and DR2 negative ATB and ITB patients and controls. A marked inversional correlation with antibody titre on spontaneous lymphoproliferative response was seen in HLA-DR2 positive ATB patients. On the other hand, a direct correlation was seen with HLA-DR2 negative patients with active disease (Fig. 3). A similar trend on the inversional correlation between antibody titre and *M. tuberculosis* culture filtrate antigen induced lymphocyte response (^{3}H-thymidine uptake - cpm) was also evident with -DR2 positive and -DR2 negative ATB patients (Fig. 4a). A similar picture was also seen with -DR2 positive and -DR2 negative control and cured patients (data not shown). However, such an inversional correlation was not observed with antibody titre and stimulation indices (SIs) of lymphocyte response to *M. tuberculosis* in HLA-DR2 positive and -DR2 negative ATB patients (Fig. 4b). On the other hand, the antibody titre and
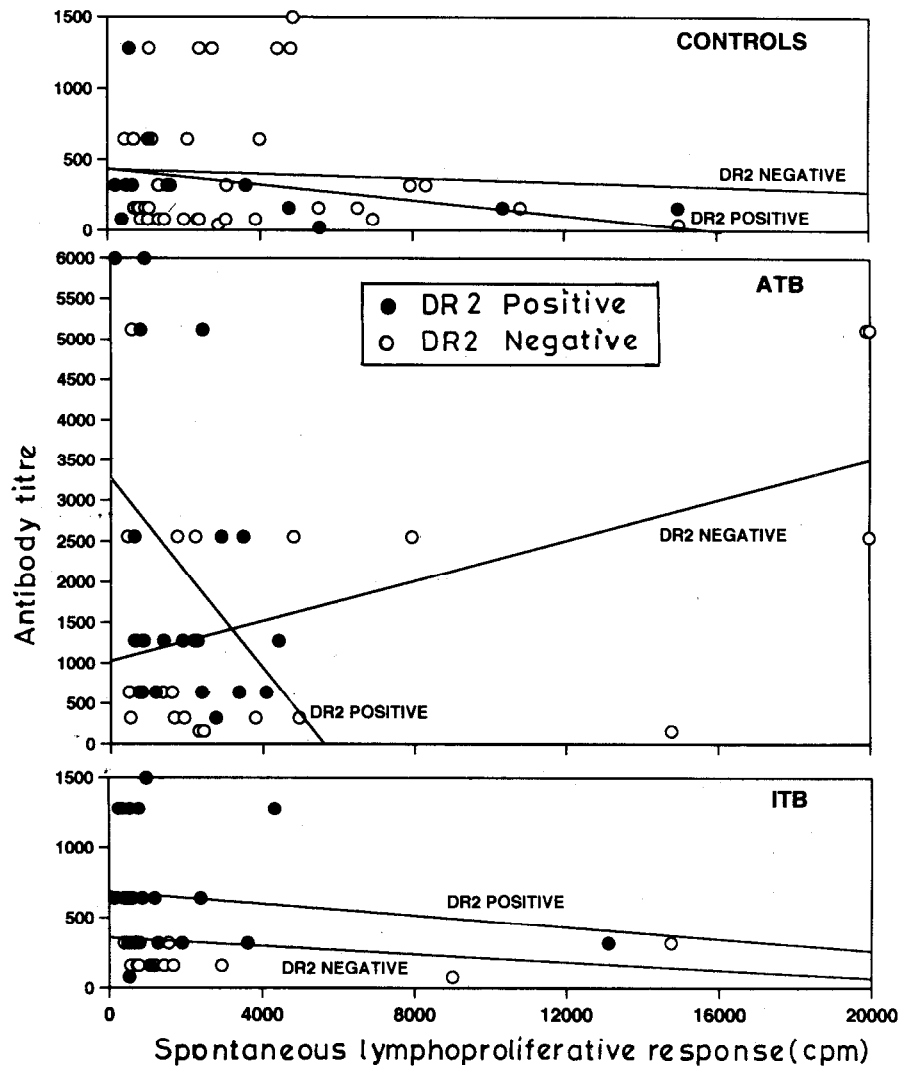
Fig. 3. Influence of antibody titre on spontaneous lymphoproliferative response in HLA-DR2 positive and -DR2 negative control subjects, active-TB and inactive-TB patients. *(i)* Control subject (-DR2 negative) and inactive-TB patient (-DR2 positive) showing antibody titres of 2560 are represented as 1500. *(ii)* Two active-TB patients (-DR2 positive) showing antibody titres of 10,240 are represented as 6000. *(iii)* Active-TB patients (3) (-DR2 negative) showing cpm of 32,008, 57,532 and 40,540 are represented as 20,000 cpm.

Discussion

An increased antibody titre to *M. tuberculosis* culture filtrate antigen was seen in HLA-DR2 positive active TB and cured TB patients. Similar increase in antibody levels against *M. tuberculosis* antigens has

the stimulation indices of the antigen induced lymphocyte response were directly correlated with HLA-DR2 and negatively correlated with non-DR2 patients with active tuberculosis (Fig. 4b).

been shown in HLA-DR2 positive Russian PTB patients[12]. This suggests that HLA-DR2 may be associated with humoral immune response. However, in the present study, a significant increase in antibody titre was not seen in DR2+ve active TB patients. This suggests that the influencing effect of -DR2 on antibody response may be regulated/masked by other factors released by monocytes/macrophages, lymphocytes and / or other cells. Suppressive effect of monocytic and lymphocytic factors on immune
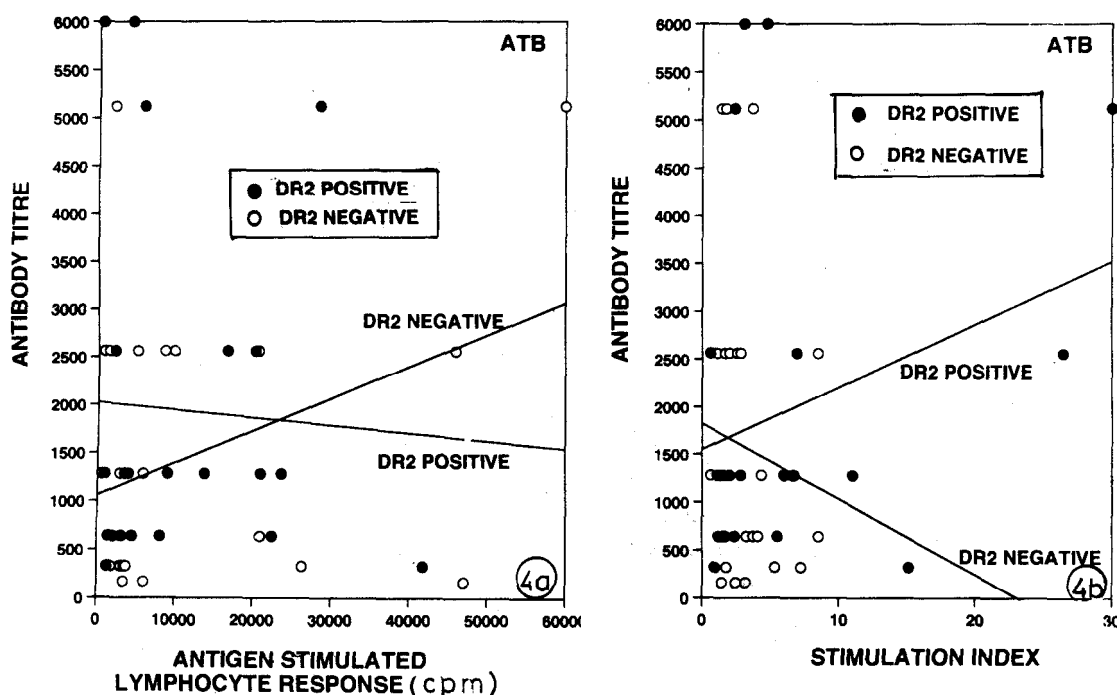
Fig. 4a. Influence of antibody titre on *M. tuberculosis* culture filtrate antigen (10 µg/ml) stimulated lymphocyte response (^{3}H-thymidine uptake-cpm) in HLA-DR2 positive and -DR2 negative ATB patients. b. Influence of antibody titre on stimulation indices (SIs) of lymphocyte response to *M. tuberculosis* culture filtrate antigen (10 µg/ml) in HLA-DR2 positive and -DR2 negative ATB patients. *(i)* -DR2 positive ATB patients (n = 2) showing antibody titres of 10,240 are represented as 6000 in Fig. 4a&b. *(ii)* - DR2 negative ATB patient (n=1) showing a cpm of 82592 is represented as 60,000 in Fig. 4a. *(iii)* -DR2 positive ATB patient (n = 1) showing a SI of 36.1 is represented as 30 in Fig. 4b.

response has been shown in PTB patients[21,22]. Such an immunoregulatory effect on antibody response is not seen in cured patients. This suggests that the inhibitory effect of the suppressive factors may not be present in the cured patients.

The immunoblot analysis revealed a trend towards an increased recognition of 38, 32/34, 30/31 kDa antigens by -DR2 positive patients (ATB and ITB) than -DR2 negative patients. It has been shown that *M. tuberculosis* 38 kDa antigen is HLA-DR2 restricted. Two of 38 kDa antigen epitopes (TB71 and TB72) are -DR2 restricted. Higher antibody to these epitopes has been shown in Indonesian PTB patients positive for HLA-DR15, a sub-type of -DR2[5,11]. In the present study a trend towards an increase in the 38 kDa recognition by HLA-DR2 positive patients (ATB and ITB) was seen. Moreover, 38 kDa antigen was intensely recognised by HLA-DR2 positive active TB patients than non-DR2 patients. This suggests that higher antibody levels

may be produced by -DR2 positive patients during the active stage of the disease. This indicates that the 38 kDa antigen as such may not be HLA-DR2 restricted, probably few epitopes may be HLA-DR2 restricted. The present study also reveals that a few of the 32/34 30/31 kDa antigenic determinants of *M. tuberculosis* may be restricted to HLA-DR2. However, this needs further study.

The spontaneous lymphoproliferative response (without antigen stimulation) and antigen induced lymphocyte response (as measured by ^{3}H thymidine uptake : cpm) were low in -DR2 positive active TB patients than - DR2 negative patients. However, the basal antibody titre to *M. tuberculosis* antigens was higher in -DR2 positive ATB patients than -DR2 negative patients. On the other hand, an increased spontaneous lymphoproliferative and antigen induced lymphocyte response (cpm) and an increased basal antibody titre were seen in non-DR2 (-DR2 negative) patients with active stage of the disease. This suggests

that the inverse correlation between antibody titre and spontaneous lymphoproliferative response and antigen induced lymphocyte response (cpm) may be associated with HLA-DR2 antigens in active TB patients.

Higher antibody titre to *M. tuberculosis* culture filtrate antigens in tuberculous sera has been shown to be inversely correlated with blastogenic responses[23]. Moreover, it has been shown that immunoglobulin G (IgG) antibody levels against *M. tuberculosis* antigen 85 complex (30, 31 and 31.5 kDa) in pulmonary tuberculosis patients were also inversely correlated with lymphoproliferation and IFN- γ secretion[24]. However, our recent studies on antibody titre and spontaneous tlastogenic response and *M. tuberculosis* culture filtrate antigen induced lymphoblastogenic response revealed no inversional correlation (unpublished observations).

Interestingly, a trend towards an inversional correlation between antibody titre and spontaneous blastogenic response and antigen induced lymphocyte response (cpm) were seen in HLA-DR2 positive active PTB patients than non-DR2 patients. However, such a down regulating effect of antibody titre was not seen on stimulation indices of *M. tuberculosis* culture filtrate antigen induced lymphocyte response in HLA-DR2 positive active PTB patients. The stimulation index of the antigen induced lymphocyte response is directly proportional to antibody titre in HLA-DR2 positive ATB patients. Whereas, a decreased antibody titre and a low stimulation index in non-DR2 (DR2 negative) patients is also directly related but showed a negative correlation. Since, stimulation index is a proportional increase (relative increase) to the spontaneous response, the picture on stimulation index represented an increased lymphocyte response to the antigen and is associated with HLA-DR2 and a decreased response with non-DR2 (DR2-negative) ATB patients. In HLA-DR2 positive ATB patients, the decreased spontaneous as well as antigen induced lymphocyte response (cpm) observed during the active stage of the disease, may be due to higher level of *M. tuberculosis* antibody and/or the components of *M. tuberculosis* (proteins, lipids and carbohydrate moieties) which may affect the antigen presentation by macrophages to T lymphocytes either by binding with the processed

antigens and/or binding with various receptors of T cells and blocking and effective antigen recognition.

In Southern Asian Indian patients with PTB, DR2 was more strongly associated with far-advanced, smear positivity cases than with cases of minimal and moderate radiographic lung lesions[7]. Further, extensive disease occurred significantly more frequently in patients with drug-failure in whom DR2 was overwhelmingly present[10]. This suggests that, during the initial stage of active tuberculosis a better cell mediated immune response may be mounted against *M. tuberculosis* in both HLA-DR2 positive and -DR2 negative patients. Since HLA-DR2 is associated with far advanced disease[7], the factors that are released by the *M. tuberculosis* infected monocyte/macrophage may suppress the immune system and permit the bacilli to grow which may lead to advanced disease. During the stage of advanced disease, the bacillary load will be high and may produce more secretory, excretory protein antigens which trigger the system to higher antibody production. At the same time cell wall components other than proteins which consist of lipids and carbohydrates *etc.,* may also be released both from live and dead bacilli into the system which may affect the antigen presentation by macrophages and antigen recognition by T-cells.

The other possibility for this decreased spontaneous and antigen induced lymphocyte (cpm) response may be due to the factors/cytokines that are released by the infected monocytes/macrophages which may regulate the cell mediated as well as humoral immune response. During the initial stage of active tuberculosis, the bacillary load may be less and a better cell-mediated immune response may be seen, when the bacillary load increases during moderate and advanced disease the CMI response may be over come by humoral immune response which may result in anergy to *M. tuberculosis* antigens and an increased antibody titre. Based on the cytokine secretion pattern by the T-cell subsets, T-helper type 1 (Th 1) (associated with CMI response), T-helper type 2 (Th2) (associated with humoral immune response), and mixed T-helper type 0 (Th0) (associated with both humoral and CMI response), have been established in mycobacterial diseases[25-27]. Recently, HLA-DR polymorphism has

been shown to modulate the cytokine profile to *M. leprae* heat shock proteins[26]. It has been suggested that the major histocompatibility complex phenotypes of the antigen presenting cell can modulate Thl-like versus Th2-like activity against *M. leprae* pathogens in leprosy and healthy individuals[26]. This suggests that the association of HLA-DR2 on tuberculo-immunity may be regulated by various cytokines released by different sub-sets of T-helper cells (Th1, Th2 and/or Th0) and/or other factors that are released by the monocytes and/or lymphocytes during the active stage of the disease.

Moreover, molecular subtyping of HLA-DR2 revealed that HLA DRBI * 1501 and *1502[10] and DRBI * 1501 (Pitchappan RM, unpublished) are associated with pulmonary tuberculosis in north Indian and south Indian patients respectively. Further studies on the influence of HLA-DR2 subtypes on tuberculo-immunity may substantiate the mechanism of disease susceptibility and the pathogenesis of the disease. The present study suggests that HLA-DR2 genes/gene products either alone or in combination with other major histocompatibility complex (MHC) genes may influence both humoral and cell mediated immune responses. However, further functional studies are needed to substantiate our data.

Acknowledgment

References

1. Brodsky FM, Guagliardi LE. The cell biology of antigen processing and presentation. *Annu Rev Immunol* 1991; *9* : 707-34.

2. Bendtzen K, Morling N, Fomsgaard A, Sevenson M, Jakobsen B, Odum N. *et al.* Association between HLA-DR2 and production of tumour necrosis factor alpha and interleukin- 1 by mononuclear cells activated by lipopolysaccharide. *Scand J Immunol* 1988; *28* : 599-606.

3. Cox RA, Downs M, Neimes RE, Ognibene AJ, Yamashita TS, Ellner JJ. Immunogenetic analysis of human tuberculosis. *J Infect Dis* 1988 *: 158 :* 1302-8.

4. Mustafa AS, Lundin KEA, Oftung F. Human T cells recognize mycobacterial heat shock proteins in the context of multiple HLA-DR molecules : studies with healthy subjects vaccinated with *Mycobacterium bovis* BCG and *Mycobacterium leprae. Infect Immun* 1993; *61* : 5294-301.

5. Bothamley GH, Beck JS, Schreuder GMT, D'Amaro J, de Vries RRP, Kardjito T, *et al.* Association of tuberculosis and *M. tuberculosis* - specific antibody levels with HLA. *J Infect Dis* 1989; *159* : 549-55.

6. Singh SPN, Mehra NK, Dingley HB, Pande JN, Vaidya MC. Human leukocyte antigen (HLA)-linked control of susceptibility to pulmonary tuberculosis and association with HLA-DR types. *J Infect Dis* 1983; *148* : 676-81.

7. Brahmajothi V, Pitchappan RM, Kakkanaiah VN, Sashidhar M, Rajaram K, Ramu S, *et al.* Association of pulmonary tuberculosis and HLA in south India. *Tubercle* 1991; *72* : 123-32.

8. Selvaraj P, Reetha AM, Uma H, Xavier T, Janardhanam B, Prabhakar R, *et al.* Influence of HLA-DR and -DQ phenotypes on tuberculin reactive status in pulmonary tuberculosis patients. *Tubercle Lung Dis* 1996; *77* : 369-73.

9. Selvaraj P, Uma H, Reetha AM, Kurian SM, Xavier T, Prabhakar R, *et al.* HLA antigen profile in pulmonary tuberculosis patients and their spouses. *Indian J Med Res* 1998; *107* : 155-8.

10. Rajalingam R, Mehra NK, Jain RC, Myneedu VP, Pande JN. Polymerase chain reaction based sequence-specific oligonucleotide hydridisation analysis of HLA-class II antigens in pulmonary tuberculosis : Relevance to chemotherapy and disease severity. *J Infect Dis* 1996; *173* : 669-76.

11. Bothamley GH, Schreuder GMT. Human leukocyte antigen, tuberculosis and *Mycobacterium tuberculosis* -specific antibody [letter]. *J Infect Dis* 1992; *165* : 598.

12. Khomenko AC, Litvinov VI, Chukanova VP, Pospelov LE. Tuberculosis in patients with various HLA phenotypes. *Tubercle* 1990; *71* : 187-92.

13. Selvaraj P, Kannapiran M, Reetha AM, Uma H, Xavier T, Narayanan PR. HLA-DR2 phenotype and plasma lysozyme, ß-glucuronidase and acid phosphatase levels in pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1997; *1* : 265-9.

14. Boyum A. Isolation of leukocytes from human blood. *Scand J Clin Lab Invest* 1968; *21* Suppl *97* : 31-50.

15. Subramanian VS, Selvaraj P, Narayanan PR, Prabhakar R, Damodaran C, Chandrasekharan P. HLA-DR and DQ antibodies in the sera of south Indian parous women. *Indian J Foren Sci* 1992; *6* : 109-13.

16. Terasaki I, McClelland JD. Microdroplet assay of human serum cytotoxin. *Nature* 1964; *204* : 998-9.

17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent ; *J Biol Chem* 1951; *193 :* 265-75.

18. Laemmli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 1970; *227 :* 680-5.

19. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets : Procedure and some applications. *Proc Natl Acad Sci (USA)* 1979; *76* : 4350-4.

20. Collins FM, Lamb JR, Young DR. Biological activity of protein antigens isolated from *Mycobacterium tuberculosis* culture filtrate. *Infect Immun* 1988; *56* : 1260-6.

21. Kleinhenz ME, Ellner JJ. Antigen responsiveness during tuberculosis : regulatory interactions of T cell subpopulations and adherent cells. *J Lab Clin Med* 1987; *110 :* 31-40.

22. Lombardi G, Di Massimo AM, Del Gallo F, Vismara D, Piccolella E, Pugliese O, *et al.* Mechanism of action of an antigen nonspecific inhibitory factor produced by human T cells stimulated by MPPS and PPD. *Cell Immunol* 1986; *98* : 434-43.

23. Havlir DV, Wallis RS, Boom WH, Daniel TM, Chervenak K, Elmer JJ. Human immune response to *Mycobacterium tuberculosis* antigens. *Infect Immun* 1991; *59* : 665-70.

24. Huygen K, Van Vooren JP, Turneer M, Bosmans R, Dierckx P, De Bruyn J. Specific lymphoproliferation, gamma interferon production and serum immunoglobulin G, directed against a purified 32 kDa mycobacteria1 protein antigen (P32) in patients with active tuberculosis. *Scand J Immunol* 1988; *27* : 187-94.

25. Misra N, Murtaza A, Walker B, Narayan NPS, Misra RS, Ramesh V, *et al.* 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 *Mycobacterium leprae. Immunology* 1995; *86* : 97-103.

26. Mitra DK, Rajalingam R, Taneja V, Bhattacharyya BC, Mehra NK. HLA-DR polymorphism modulates the cytokine profile of *Mycobacterium leprae* HSP-reactive CD4+T ceils. *Clin Immunol Immunopathol* 1997; *82* : 60-7.

27. DiPiro JT. Cytokine networks with infection : mycobacterial infections, leishmaniasis, human immunodeficiency virus infection and sepsis. *Pharmacotherapy* 1997; *17 :* 205-23.

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