Original Article

HLA-DR PHENOTYPES AND LYMPHOCYTE RESPONSE TO *M. TUBERCULOSIS* ANTIGENS IN CURED SPINAL TUBERCULOSIS PATIENTS AND THEIR CONTACTS

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Summary

Background: Our earlier studies on Human Leucocyte Antigens (HLA) in pulmonary tuberculosis patients revealed the association of HLA-DR2 antigen with susceptibility to pulmonary TB and DR2 antigen has been shown to influence the immunity to tuberculosis.

Objectives: The present study was carried out to find out whether HLA-DR antigens are associated with susceptibility to spinal tuberculosis. Moreover, the role of HLA-DR antigens on lymphoproliferative response to *Mycobacterium tuberculosis* culture filtrate antigens was studied using Lymphocyte Transformation Test (LTT).

Material and Methods: HLA-DR genotyping and lymphoproliferative response was carried out in 63 cured spinal TB patients and 63 control subjects (spouses of pulmonary and spinal TB patients).

Results: A trend towards an increased frequency of HLA-DR9 antigen was observed in spinal TB patients compared to controls. A significantly decreased lymphocyte response to *M. tuberculosis* antigens was observed in HLA-DR9 antigen positive control subjects compared to HLA- DR9 antigen negative subjects (P=0.0009) whereas increased response was observed with DR9 positive cured spinal TB patients compared to HLA-DR9 antigen negative patients. Further, HLA-DR3 antigen positive patients showed a decreased lymphocyte response compared to HLA-DR3 antigen negative patients ($P<0.05$).

Conclusion: The study suggests that HLA-DR9 antigen either alone or in combination with other HLA antigen as lhplotype and non-HLA genes may be associated with susceptibility to spinal TB and play a regulatory role on the immune response to *M. tuberculosis* in spinal tuberculosis patients. **[*Indian J Tuberc 2004; 51:71-75*]**

Key Words: HLA-DR, lymphocyte response, *M. tuberculosis antigen*, cured spinal tuberculosis

INTRODUCTION

Spinal tuberculosis, a degenerative disease of the spine caused by *Mycobacterium tuberculosis*, also known as Pott's disease, usually occurs due to lymphohaematogenous spread from a primary focal area, such as the lung. It may also result from direct invasion of a paravertebral focus by lymphangitic spread from paravertebral lymph nodes or the pleural space[1,2]. Our earlier studies in pulmonary tuberculosis revealed the association of HLA-DR2 antigen with the susceptibility to pulmonary tuberculosis[3]. Our further studies also revealed the influence of HLA-DR2 antigen on antibody titre, spontaneous lymphoproliferative response and antigen induced lymphocyte response including memory response in pulmonary tuberculosis[4,5].

Earlier, we have studied the humoral and lymphocyte response to *M. tuberculosis* antigens in cured spinal tuberculosis patients and the immune response pattern was compared with the contacts[6]. The present study was carried out to find out whether HLA-DR2 antigen is associated with the susceptibility to spinal tuberculosis. Moreover, the influence of HLA-DR genes on lymphoproliferative response to *M. tuberculosis* antigens was studied to understand the memory response, if any, in cured spinal TB patients.

MATERIAL AND METHODS

Spinal tuberculosis patients: Patients included in this study were cured spinal tuberculosis patients who were treated 15-20 years ago. A total

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of 63 patients were studied, of which 39 were females (mean age ± standard error (SE) = 45.7±2.2 yrs) (range 21-78 yrs) and 24 were males (mean age±SE = 40.0±3.3 yrs) (range 21 -74 yrs). These patients were selected from subjects of an earlier chemotherapy study carried out at our centre[7]. Clinically and radiographically active spinal tuberculosis patients involving any vertebral body from the first thoracic to the first sacral were admitted to the chemotherapy study. Patients were excluded if they had (a) paralysis of the lower limb severe enough to prevent them from walking (b) serious extraspinal disease, tuberculous or non-tuberculous (c) history of previous chemotherapy for 12 months or more, or (d) already had major surgery for their spinal tuberculosis. All the patients were in quiescent stage at the time of blood sample collection for the present study.

Controls: Normal healthy spouses of the spinal tuberculosis patients and pulmonary tuberculosis patients were studied. Of the 63 contacts studied, 39 were females (mean age ± SE = 36.5 ± 1.5 yrs) (range 20-60 yrs) and 24 were males (mean age ± SE = 43.4 ± 2.7 yrs) (range 23 -65 yrs). The contacts were clinically normal at the time of blood sample collection. The spouses were living together with the patients before, during and after treatment for about 15-20 years. The patients and the controls belonged to the same ethnic origin, socio-economic status and were not consanguineous.

Lymphoproliferative response to Mycobacterium tuberculosis antigen: Peripheral blood mononuclear cells (PBMC) were separated from heparinised (20 units/ml) blood samples using Ficoll-Hypaque density gradient centrifugation[8]. Lymphocyte transformation test (LTT) to culture filtrate antigen of *M. tuberculosis* (10 μg/ml) was carried out as described in our earlier study[4].

Extraction of human genomic DNA: DNA was extracted from peripheral blood white cells using a salting out procedure[9].

Genotyping of HLA-DR: DNA typing of HLA-DR was done using a combination of Group Specific Amplifications (GSA), Amplified Fragment Length Polymorphism (AFLP) and hybridization with Sequence Specific Oligonucleotide Probes (SSOP), which involved two sequential steps: first, GSA for six groups of HLA-DR generic types with group specific primers: DR1, DR2, DR4, DR10, DR7 and DR9 and finally DR3, DR5, DR6, DR8. At the end of the first step DR1, DR2, DR4 and DR10 were assigned directly based on the presence or absence of amplified polymerase chain reaction (PCR) product, DR7 and DR9 were assigned by AFLP after digestion of amplified DNA with the endonuclease *Hinf*I[10]. Second, DNA typing of DR3, DR5, DR6 and DR8 in the second exon of the DRB gene was done by group specific amplification using a set of primers and then by using SSOP to assign the generic types[11].

Statistical Methods: The frequencies of HLA-DR antigens in patients and contacts were determined by direct allelic count and expressed as percent phenotype (% PF). 2x2 Chi-square test (χ^2) was performed for ascertaining the significance of the HLA antigens. The P value (with Yates' correction) was determined to find out the significance. Relative risk (RR) was calculated using the appropriate formula[12]. The results on the lymphoproliferative response was analysed using Student's t test and the significance was determined. Results of the lymphoproliferative response are expressed as arithmetic mean ± standard error (S.E.). P value less than 0.05 was considered significant.

RESULTS

Among the various HLA-DR antigens, increased antigen frequency and relative risk were observed with HLA-DR9 in spinal TB patients as compared to control subjects (DR9: Spinal TB 12.7%; Control 3.2%) (Relative Risk DR9: 1.7). The antigen frequency of HLA-DR2 was similar to that of controls [spinal TB : 31.7%; control: 30.2%] (Table).

The lymphoproliferative response to *M. tuberculosis* antigen of cured spinal TB patients was similar to control subjects. The Stimulation Index (SI), of controls was 9.0 ± 1.4, whereas the SI was 9.7 ± 1.6 in cured spinal TB patients. Moreover, no difference was observed irrespective of the DR

Table: Percentage phenotype frequencies of HLA-DR antigens in spinal TB patients and controls

HLA ANTIGENS	% Phenotype frequency	
	Controls (n=63)	Spinal TB (n=63)
HLA-DR		
DR 1	12.7 (8)	19 (12)
DR 2	30.2 (19)	31.7 (20)
DR 3	20. 6 (13)	14.3 (9)
DR 4	28.6 (18)	20.6 (13)
DR 5	25.4 (16)	22.2 (14)
DR 6	33.3 (21)	30.2 (19)
DR 7	28.6 (18)	31.7 (20)
DR 8	9.5 (6)	4.8 (3)
DR 9	3.2 (2)	12.7 (8)
DR 10	7.9 (5)	12.7 (8)*
DR blank	0	0

n = Number of individuals studied; numbers in parentheses represent the individuals positive for each antigen. * HLA-DR9 χ^2 uncorrected p=0.048 (significant). Relative Risk: DR9:1.7

phenotype of the patients and control subjects. HLA-DR9 positive control subjects showed a significantly low lymphocyte response to *M. tuberculosis* culture filtrate antigens (Control : DR9 positive vs DR9 negative : P=0.0009). An increased lymphocyte response was observed in -DR9 positive cured spinal TB patients than -DR9 negative patients. Spinal TB patients positive for HLA- DR3 and HLA-DR10 antigens showed a decreased lymphocyte response to *M. tuberculosis* antigens (DR3 positive vs DR3 negative : P<0.05; DR10 positive vs DR10 negative : P=0.002) when compared to HLA-DR3 and HLA-DR10 negative patients (Figure).

DISCUSSION

In the present study, a trend towards an increased antigen frequency of HLA-DR9 (12.7%) was observed with the spinal TB patients compared to control subjects (DR9 : 3.2%) (DR-9 Relative Risk : 1.7). Further, in the present study, HLA-DR2

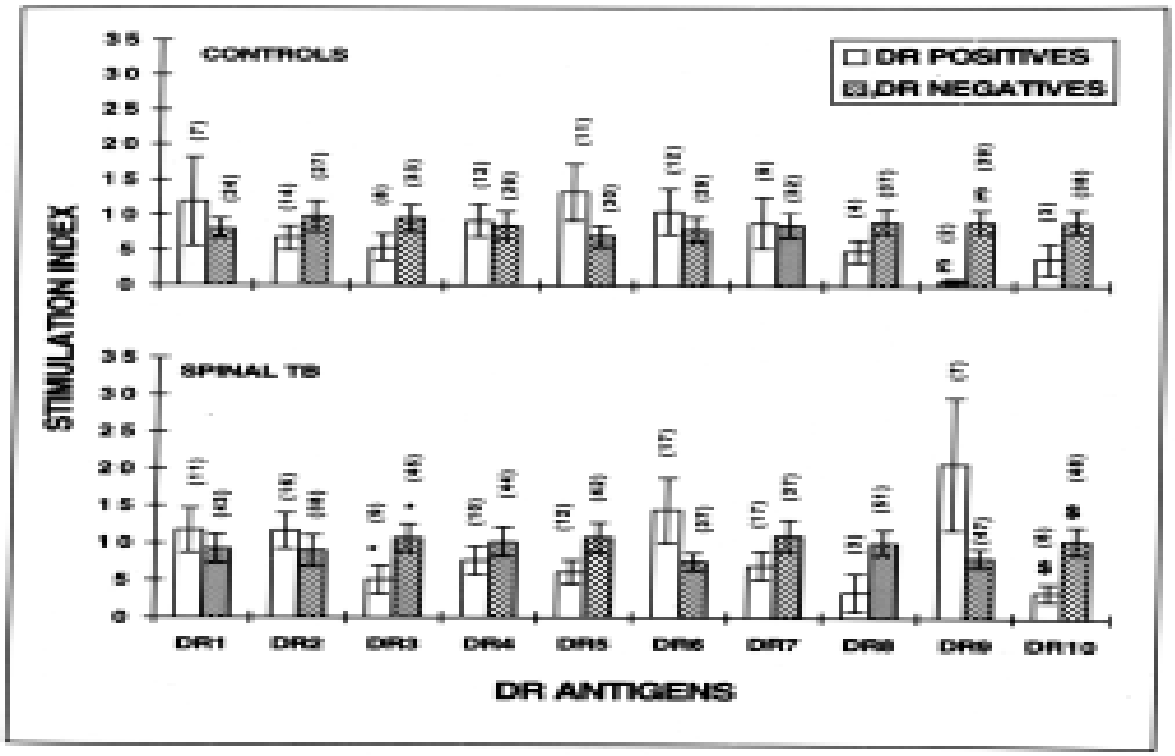

Figure: Influence of HLA-DR antigens on lymphocyte response to *M. tuberculosis* culture filtrate antigens (10 μg/ml) in cured spinal TB patients and controls. Results are expressed as arithmetic mean ± S.E of the stimulation index. Numbers in parentheses represent the subjects studied.
£P=0.0009;*P<0.05;$P=0.002

(control : 30.2%; Spinal tuberculosis : 31.7%) was not found to be associated with the susceptibility to spinal TB, whereas our earlier study showed the association of HLA-DR2 (Control: 29.5%; pulmonary TB : 48.8%) with the susceptibility to pulmonary TB. In south Indian patients with pulmonary TB, DR2 has been shown to be more strongly associated with far advanced, smear positive cases than with cases with minimal and moderate radiographic lung lesions[13]. On the other hand, spinal tuberculosis is a localized disease with paucibacillary state. Our recent study on vitamin D receptor gene (non-HLA gene) polymorphism (*Bsm*I, *Apa*I, *Taq*I & *Fok*I polymorphism), revealed the association of the genotypes Bb (heterozygotes) of *Bsm*I polymorphism and FF (homozygotes of frequent allele 'F') of *Fok*I polymorphism of vitamin D receptor gene with the susceptibility to spinal tuberculosis[14]. **Probably HLA-DR9, either alone or in combination with other HLA antigen as haplotype and non-HLA genes, may be associated with paucibacillary disease such as spinal tuberculosis.**

The lymphoproliferative response to *M. tuberculosis* culture filtrate antigens of cured spinal TB patients did not differ from control subjects. This suggests that normal immune response is restored in cured spinal TB patients and is similar to that of normal subjects. HLA-DR9 antigen positive control subjects showed a decreased proliferative response to *M. tuberculosis* antigens compared to - DR9 antigen negative control subjects. An increased response with -DR9 positive patients was observed as compared to -DR9 negative patients. This suggests that HLA-DR9 antigen may be associated with low responder status to *M. tuberculosis* infection which may probably allow the pathogen to establish the infection in a susceptible DR9 positive normal subjects and associated with increased lymphoproliferative response during disease state.

In the present study, cured spinal TB patients positive for HLA-DR3 and -DR10 antigens showed a significantly decreased lymphocyte response to *M. tuberculosis* antigens. HLA- DR3 associated non-responder status of T-lymphocyte responses to PPD has been reported[15]. The decreased response associated with DR3 has been suggested to be due to decreased IL-2 production[16]. A similar mechanism may also be possible for the lower lymphocyte response observed in DR3 and DR-10 positive cured patients.

The present study suggests that HLA-DR9 either alone or in combination with other HLA antigens as haplotype and non-HLA genes such as vitamin D receptor gene may be associated with susceptibility to spinal tuberculosis. Further, HLA-DR9 may also play a regulatory role on the immunity to *M. tuberculosis* in spinal tuberculosis.

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FOR AUTHORS

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Journal

Jain NK, Chopra KK, Prasad G. Initial and Acquired drug resistance to Isoniazid and Rifampicin and its implications for treatment. *Indian J Tuberc* 2002; **39**: 121-124.

Book

Crofton J, Douglas A. *Respiratory Disease*; Ist Edn. Edinburgh: Blackwell Scientific Publications Ltd.,1969.

Chapter in Book

Fraser RS, Muller NL, Colman N, Pare PD. Upper airway obstruction. *In*: Fraser RS, Muller NL, Colman N, Pare PD, Bralow L, ed Fraser and Pare's *Diagnosis of Diseases of Chest*; 4th edn; Vol III. Philadelphia: W.B. Saunders Co.; 1999: 2021-2048.

What To Do For Quitting Smoking

After making a firm resolve to quit smoking, you may take the following steps :

1. Consult your doctor. He is bet placed to show you the way and help you medically at cruical junctures.

2. Join or form a group/an association of smokers who have successfully quitted, like the Alcoholics Anonymous for drinkers.

3. Read guide-book about quitting smoking.

4. Keep trying instead of thinking how difficult it is to quit or the pleasure you might get from just a single cigarette.

5. Talk freely to other smokers about how you are already succeding. And advise the vulnerable non-smokers why they should never start the habit. This activity will help boost your own morale.

6. Finally, have full faith in your own self. You are the one who is going to succeed. Do not deprive yourself of some therapies that are available for ‘nicotine replacement’, if your doctor so advises.

YOU HAVE TO QUIT

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