ASSOCIATION OF HLA-CLASS I ANTIGENS AND HAPLOTYPES WITH RELAPSE OF PULMONARY TUBERCULOSIS IN PATIENTS TREATED WITH SHORT COURSE CHEMOTHERAPY

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Summary **: Whether or not there is an association between HLA antigen(s) and/or haplotypes aud relapse in patients successfully treated for pulmonary tuberculosis was examined. Serological determination of HLA -A, - B, -DR and -DQ antigens was carried out in patients with quiescent pulmonary tuberculosis and bacteriologically relapsed patients, after treatment with short course chemotherapy with Rifampicin, Isoniazid, Pyrazinamide and Streptomycin or Ethambutol in various combinations for 6-8 month Au increased antigen frequency of HLA -Al (P = 0.03) and B17 was seen in patients with bacteriological relapse compared with those with quiescent disease. The relative risks (RR) were Al = 2.8 and B17 = 3.2, respectively, The haplotypes Al-B17 (RR = 3.3), B17-DR7 (RR = 3.0), Al-DR7 (p = 0.04; RR = 9.3) were very common in patients with bacteriological relapse. This increase of HLA-Al, B17 antigens or the haplotypes Al-B 17, B17-DR7 or Al-DR7 (P = 0.04) was seen irrespective of the treatment regimen. The present study suggests that HLA -Al (and -B17) antigen(s), as such, and/or haplotypes Al-DR7 or non-HLA genes linked closer to HLA -A, -B and -DR loci may be associated with relapse of pulmonary tuberculosis, after chemotherapy.**

INTRODUCTION

Pulmonary tuberculosis is a granulomatous lung disease caused by *M. tuberculosis.* Treatment with short course chemotherapy with Rifampicin containing drug regimens has proved to be highly effective[1,2]. Inspite of successful treatment with these drugs, a proportion of patients relapse, which requires retreatment[2,3]. These relapses may be either due to "endogenous reactivation", where the dormant forms of the organism (persisters) start multiplying again, after completing the treatment, due to the trigger provided either by the host factors and/or environmental factors. The other reason suggested for relapse is "exogenous reinfection"[4,5].

It is well established that the immune status of the host is regulated by HLA genes/gene products which play a major role in cell-mediated and humoral immune responses to any pathogen or antigen[6].

The present study was carried out to understand the association, if any, between HLA genetic factors and relapse of pulmonary tuberculosis, after successful treatment with short course chemotherapy.

MATERIAL AND METHODS

Study Subjects

For the study, 51 patients who had a bacteriological relapse after 6-8 months' treatment with short course chemotherapy were selected. An equal number of patients with quiescent disease, matched for treatment regimen and duration of follow up was also selected. The primary treatment regimen consisted of Rifampicin, Isoniazid, Pyrazinamide and Streptomycin (or Ethambutol) for the initial phase and 2 or 3 drugs in the continuation phase for 6 to 8 months. All the patients had bacilli sensitive to Streptomycin, Isoniazid and Rifampicin, initially. Of the 51 relapses, 39 had occurred in the first 12 months of

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follow up. Care was taken to select controls who were not related to the patients with relapse.

Among the 51 relapse cases, 43 were males, mean age was 33.0 with SE ± 1.8 years, and for the 8 females, mean age was 32.8 with SE ± 5.1 years. Among the quiescent cases, 35 were males, mean age was 32.1 with SE ± 1.5 years and for 16 females, mean age was 29.5 with SE ± 2.1 years.

Peripheral blood mononuclear cells

Twenty milliliters of peripheral blood in heparin (20 units/ml) was drawn from each patient. The blood specimens were subjected to ficoll-hypaque density gradient centrifugation as described by Boyum[7]. The mononuclear cells were separated and washed in TC 199 tissue culture medium (Sigma, USA) and used for HLA typing.

HLA typing

T and B lymphocytes were separated from peripheral blood mononuclear cells by a nylon wool column[8]. Eighty to hundred mg of nylon wool (Fenwall Laboratories, USA) was evenly packed in each column for 6 cm in a 10 cm column using a drinking straw. The columns were rinsed with TC 199 medium containing 10% FCS. Ten million mononuclear cells in 1 ml medium were loaded on to nylon wool and incubated at 37°C for 40 minutes. The nylon wool non-adherent cells (enriched T cells) were used for HLA -A and -B typing and the nylon wool adherent population (enriched B cells) was used for HLA -DR and -DQ typing. HLA phenotyping was performed by a two stage micro lymphocytoxicity assay[9].

The antisera for HLA -A and -B (class-I) and HLA -DR and -DQ (class-II) antigens were purchased from Biotest, Germany. In addition, well established sera of local origin[8,10] and some procured from co-operative HLA laboratories in India were also used. At least three sera were included for each specificity studied.

Statistical Analysis

The frequencies of HLA alleles, in patients and controls, were determined by direct allelic count method. A2 x 2 contingency Table was constructed and Chi-square analysis with Yates correction was performed for HLA antigens and haplotypes. Relative Risk (RR) (Odd's ratio) values were calculated to find out the strength of the association between HLA antigens and/or haplotypes and the occurrence of relapse".

RESULTS

An increased antigen frequency of HLA -Al and -B 17 was seen in patients with bacteriological relapse compared with those having quiescent disease (Al P = 0.03). A trend towards an increase in the frequency of HLA -A3, A28, B12, B40 was also seen in the quiescent patients when compared with relapse patients. However, the increase was not significant. Among the HLA -DR and -DQ loci antigens, the trend was towards an increased frequency of DR3, DR6 and DR10 and decreased frequency of DR5, DR8 and DR9 in patients with relapse. The increased or decreased DR antigen frequencies were not significant. No trend was observed for -DQ locus antigens (Table 1).

In relapse patients, the relative risk values (RR) were higher for Al (2.8), B17 (3.2) DR3 (2.5) and DR6 (2.1) antigens. Further, the haplotype frequency of Al-B17, B17-DR7, Al-DR7, Al-B8, B8-DR3, Al-DR3 were higher in the relapse patients than quiescent (controls) patients. Moreover, the relative risk values were also higher for Al-B17 (3.3), B17-DR7 (3.0) Al-DR7 (9.3) Al-DR3 (3.3) in relapse patients. The haplotype Al-DR7 was significantly increased in relapse patients (P = 0.04). An increased frequency of the haplotype B12-DR7 was seen in the quiescent patients compared with the relapse patients. However, this increase was not significant (P = 0.06).

DISCUSSION

In the present study, the antigen frequency of HLA -Al, -B17 was higher in pulmonary tuberculosis patients who had bacteriological relapse after completing SCC compared with quiescent patients. Further, the haplotype frequency of Al-B17 was also increased. Though the haplotype Al-B17 (B57) is found mainly in Caucasian populations[12], this haplotype is also seen in the Indian population[13]. The phenotype frequency of Al and B 17 antigens and the haplotype frequency of Al-B 17 was found to be higher in those Indian patients who had psoriasis[14]. Increased frequency of Al-DR7 and Al-DR3 haploytpes has

***Table 1* : HLA -A, -B, -DR and -DQ antigen frequencies in relapse and quiescent (controls) patients of pulmonary tuberculosis treated with short course chemotherapy**

HLA	% Phenotype frequency in Quiescent (n=51)	% Phenotype frequency in Relapse (n=51)	Relative Risk (RR) (Odd's ratio)
A-locus			
Al	21.6*	43.1*	2.8
A2	35.3	29.4	0.8
A3	23.5	13.7	0.5
A9	25.5	27.5	1.1
A10	15.7	15.7	1.1
A11	33.3	35.3	1.1
A19	17.6	19.6	1.1
A28	13.7	3.9	0.3
A-	15.7	11.8	–
B-locus			
B5	39.2	41.2	1.1
B7	23.5	23.5	1.0
B8	5.9	5.9	1.0
B12	11.8	3.9	0.3
B13	9.8	13.7	1.5
B14	0.0	3.9	–
B15	21.6	21.6	1.0
B17	7.8**	21.6**	3.2
B21	7.8	7.8	1.0
B22	3.9	3.9	1.0
B35	29.4	21.6	0.7
B40	21.6	9.8	0.4
B-	17.6	21.6	–
DR-locus			
DR1	11.8	7.8	0.6
DR2	49.0	52.0	1.2
DR3	9.8	21.6	2.5
DR4	29.4	21.6	0.7
DR5	21.6	13.7	0.6
DR6	5.9	11.8	2.1
DR7	29.4	37.3	1.4
DR8	7.8	2.0	0.2
DR9	11.8	5.9	0.5
DR10	9.8	15.7	1.7
DR-	13.7	9.8	–
DQ-locus			
DQ1	82.4	88.2	1.6
DQ2	33.3	41.2	1.4
DQ3	58.8	54.9	0.9
DQ-	23.5	15.7	–

* $\chi^2 = 4.48$; P = 0.03 Significant
** $\chi^2 = 2.8$; P =0.06 Non-significant

***Table 2* : Haplotype frequencies (per 10,000) in relapse and quiescent (controls) patients of pulmonary tuberculosis treated with short course chemotherapy**

Haplotypes	Haplotype frequency Quiescent	Haplotype frequency Relapse	Relative Risk (RR) (Odd's ratio)
Al - B5	348	213	1.5
Al - B8	–40	299	–
Al - B12	152	67	0.5
Al - B17	176	463	3.3
Al - DR3	164	463	3.3
Al - DR5	209	31	0.7
Al - DR7	–139*	478*	9.3
A3 - B5	319	182	0.6
A3 - DR3	160	20	0.5
A3 - DR5	69	–59	0.0
A28 - B5	318	69	0.2
A28 - DR5	66	73	0.5
B5 - DR5	207	–98	0.2
B5 - DR7	336	671	1.6
B8 - DR3	–16	187	–
B12 - DR7	487**	–53**	0.0
B17 - DR7	281	763	3.0

* $\chi^2 = 4.39$; P = 0.03 Significant
** $\chi^2 = 3.36$; P =0.06 Non-significant

been found in relapse pulmonary tuberculosis patients. It has been shown that the haplotype Al-B8-DR3 is associated with autoimmune diseases[15].

In Hong Kong Chinese tuberculosis patients, who had relapsed after 6 months or more of inactivity, no increase or decrease in any of HLA-antigens or haplotypes was seen[16]. However, the present study shows an increase in the HLA -Al and -B17 antigens and A1-B17, A1-DR7 and A1-DR3 haplotypes among similarly treated patients. The present study suggests that either the antigen HLA -A1 and/or the haplotype A1-DR7 are associated with bacteriological relapse or the non-HLA genes which are linked to the HLA region may be involved in the occurrence of bacteriological relapse in treated pulmonary tuberculosis patients.

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