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HLA-DR Antigens in Patients with Pulmonary Tuberculosis in Northern Poland. Preliminary Report

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Abstract. The aim of the present study was the analysis of the association between particular class II HLA antigens and the incidence of tuberculosis in northern Poland. HLA-DR antigens in a group of 26 patients with pulmonary tuberculosis (PTB) and 58 healthy volunteers were determined. Histocompatibility typing was performed by the PCR-SSP method using primers from the Dynal company. For statistical analysis, the χ^2 test was used with Yates' correction. The probability values were weighted for the number of antigens tested (pc). The relative risk (RR) was calculated by Woolf's method. We found that HLA-DR16(2) antigen expression was significantly higher in patients with tuberculosis than in the tested group of healthy controls ($p < 0.001$, $pc < < 0.01$); the highest relative risk (RR = 12.4) of tuberculosis incidence was connected with DR16(2) antigen, the prevalence of HLA-DR13(6) antigen was significantly lower in pulmonary tuberculosis (with RR = 0.09) than the control ($p < 0.001$, $pc < 0.01$). The results obtained suggest that the presence of HLA-DR16(2) antigen can extend the risk of developing tuberculosis whereas HLA-DR13(6) antigen occurrence was significantly more rare in pulmonary tuberculosis than in healthy individuals and that the relative risk (RR = 0.09) can be connected to their relation with the genes of insusceptibility to tuberculosis.

Key words: HLA-DR; pulmonary tuberculosis.

Introduction

Despite the fact that *Mycobacterium tuberculosis* was discovered 100 years ago and that since then there have been numerous attempts to determine the immunoreactivity of the infected organism, it is still not clear why only a small fraction of all persons infected eventually develop tuberculosis. Attempts are being made to establish a correlation of the effects of the immune answer with the occurrence of particular antigens of class II of the human leukocyte antigens (HLA) system. The HLA-DR antigens regulate host response to infection by *M. tuberculosis* through cell-mediated immunity[1, 3, 4, 5, 8, 18, 21]. A genetic predisposition to the development of tuberculosis was suggested by the reported increased incidence of this disease in twin studies[11].

Data from the literature concerning the correlation between tuberculosis and antigens of the HLA system are not consistent and indicate divergences in various ethnic groups. HAWKINS et al.[9] did not indicate such HLA-DR system association. Later papers by HAFEZ et al.[7] confirmed a relationship between the number suffering from tuberculosis and the HLA-DR antigens. No such analysis has, as yet, been carried out in Poland.

The aim of the present study was an attempt to

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answer the questions as to whether there is any connection between the HLA system locus-DR and increased morbidity in pulmonary tuberculosis.

Materials and Methods

Patients. Twenty-six persons with newly detected active pulmonary tuberculosis were studied. In this group were 14 women and 12 men ranging in age 22–68 years (mean 40.7 ± 16). The diagnosis of tuberculosis was established using standard clinical, radiological and bacteriological criteria at the Pulmonology Department of the University Medical School in Gdańsk. Also included in the studies were those patients who, having been informed of the expedience of carrying out the studies, afforded written confirmation. The studies were approved by the local the Ethicals Committee.

The control group consisted of 58 healthy, unrelated persons matched for age and sex with the patient group. In these persons, HLA antigens typing was carried out at the Department of Immunopathology of the Medical University School in Gdańsk. There was no tuberculosis in the families of this group of persons.

About 10 ml of peripheral blood was taken from each individual, leukocytes were isolated and antigens of HLA class II-DR determined.

HLA-DR typing. For the determination of the DR antigens, the PCR-SSP (polymerase chain reaction with sequence specific primer) method was performed, with a specific pair of DYNAL primers for 14 HLA-DR specificity[14].

DNA extraction. DNA was prepared by phenol/chloroform extraction. DNA from homozygous cells of 10th International Histocompability Workshop was used to verify primer specificities.

Amplification conditions. The PCR reactions were carried out in 25 μl volumes, which contained genomic DNA template, 10 pmol of each sequence-specific primer, 200 μM of each dNTP, 2 units of Taq DNA polymerase, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, and 0.1% Triton-X. Samples were amplified after initial denaturation at 94˚C for 3 min followed by 30 cycles of 94˚C denaturation for 60 s, 60˚C annealing for 90 s, 72˚C for extension for 60 s and a final 72˚C extension for 5 min, using a thermal sequence TSR-300.

Statistical analysis. To compare frequency between study populations chi-squared test, incorporating Yates' correction, was used[17]. The probability values were corrected for the number of antigens tested (pc) by multiplying them by the number of antigens tested. A pc value of 0.05 or less was considered to be significant. The upper limit was defined as the mean ($\overline{x}$) ± 2SD (standard deviation) for each tested parameters. The relative risk (RR) was defined based on Woolf's method[22].

Results

The frequencies of HLA-DR antigens in the group of 26 patients with pulmonary tuberculosis and 58 control individuals are presented in Table 1.

The results indicate that DR16(2) antigen was detected more frequently in persons with tuberculosis than in the control. This antigen was noted in 31% of the patients and 3% of the healthy subjects ($p < 0.001$; $pc < 0.01$), whereas HLA-DR15(2) antigen expression was comparable with the control persons. Significantly rare in the group of patients (4%) was HLA-DR13(6) antigen, which occurred in 29% of the healthy individuals ($p < 0.001$) and was significant when corrected p values were applied ($pc < 0.01$). The HLA-DR14(6) antigen was noted with a similar frequency in both populations studied.

In patients with HLA-DR16(2) antigen in phenotype, increased values of relative risk (RR = 12.4) are found whereas a low relative risk (RR = 0.09) of HLA--DR13(6) antigen incidence in the same group of individuals was observed.

A comparison of frequency of occurrence for other specifities of HLA antigens tested in both populations failed to show essential statistical differences and, as regards HLA-DR4, -DR14(6), -DR7 and -DR8 antigens, a higher value (>1) of relative risk of the presence tuberculosis was noted.

Discussion

One of the first reports of an association between the HLA-class I antigens and tuberculosis was that by SELBY et al.[19] Thus, the association of tuberculosis with HLA class II antigens may be more relevant than that with HLA-A, B, C antigens because cell-mediated immunity is known to be involved in the pathogenesis of tuberculosis.

There have been few reports about HLA-DR antigens in tuberculosis. One earlier study on an association between HLA-DR antigens and tuberculosis showed an increase in DR5 in the Egyptian population[7]. At the same time, significantly increased frequencies for HLA-DR5 in Americans black patients have been re-

Table 1. Phenotype frequency of HLA-DR antigens in patients with pulmonary tuberculosis (PTB) compared with frequency in healthy controls

Type of antigen of class II HLA system	Controls (n = 58)		PTB patients (n = 26)		RR	χ^2	p	pc
	pos.	%	pos.	%				
DR 1	9	15	2	8	0.45	1.7	NS	NS
DR 15(2)	15	26	2	8	0.23	3.6	NS	NS
DR 16(2)	**2**	**3**	**8**	**31**	**12.4**	**10.3**	**<0.001**	**<0.01**
DR 17(3)	15	26	5	19	0.68	0.4	NS	NS
DR 18(3)	0	0	1	4	0	0.1	NS	NS
DR 4	10	1	5	19	1.14	0.01	NS	NS
DR 11(5)	16	27	6	23	0.78	0.1	NS	NS
DR 12(5)	3	5	1	4	0.73	0.6	NS	NS
DR 13(6)	**17**	**29**	**1**	**4**	**0.09**	**6.9**	**<0.001**	**<0.01**
DR 14(6)	3	5	2	8	1.52	0.002	NS	NS
DR 7	10	1	7	27	1.76	1.04	NS	NS
DR 8	4	6	2	8	1.12	0.3	NS	NS
DR 9	1	1	0	0	0	3.1	NS	NS
DR10	3	5	0	0	0	3.3	NS	NS

pos – antigen positive persons.

RR – relative risk=$\frac{\text{number of antigen positive patients} \times \text{number of antigen negative controls}}{\text{number of antigen positive controls} \times \text{number of antigen negative patients}}$.

pc – p values corrected by multiplying them by the number of antigens tested.

NS – not significant ($p > 0.05$).

ported[10]. Later, the association of tuberculosis with the antigen HLA-DR2 occurred in studies conducted by SINGH et al.[20] in the Indian Hindu population. This was confirmed by the observations of BOTHAMLEY et al.[1], HARBOE and WIKER[8], as well as KHOMENKO et al.[12], which demonstrated a close association between DR2 and elevated levels of antibody to epitopes on a 38 kDa protein specific to *M. tuberculosis*. However, the available literature does not show a DR2 connection with tuberculosis in Western white populations[2, 10].

A negative correlation (i.e., decreased antigen frequencies) between *M. tuberculosis*, a pathogenicity factor and the antigen HLA-DRw6 was disclosed by HWANG et al.[10] and SHING et al.[20]. Nevertheless, KHOMENKO et al.[12] showed a decreased frequency of the HLA-DR3 antigen in pulmonary tuberculosis. According to the authors of the paper[18], antigen DR3 and DR5 affects the regulation of the immunoreactivity of an organism to the 65 kDa protein of the tubercle bacillus.

All of these studies were based on serologic testing of expressed HLA antigens on the surface of lymphocytes, a method which, incidentally, shows a discrepancy of up to 16.4%[16] and 25% in DR typing results compared with the more sensitive molecular DNA-based methods[15].

Inconsistent results were obtained by GOLDFELD et al.[6] and RAJALINGAM et al.[16] in their studies of the association between the HLA-DR2 antigen and tuberculosis using the more sensitive polymerase chain reaction-based sequence-specific oligonucleotide probe hybridization technique (PCR-SSOP). In molecular typing, the HLA-DR2 antigen was present more frequently in Asian Indian tuberculous patients than in controls[16], whereas GOLDFELD et al.[6] did not confirm this data in the Cambodian population with tuberculosis. Likewise, a negative correlation (i.e., decreased antigen frequencies) between *M. tuberculosis*, a pathogenicity factor and the HLA-DR6 antigen was disclosed by HWANG and colleagues[10] while SHING et al.[20], did not, was confirmed in molecular typing[7, 16].

The results of our research confirmed some of the above observations and the up-to-date method adopted enabled more accurate determination of the antigens of the HLA class II system. The PCR-SSP method used in the present study showed that among those suffering from tuberculosis, the occurrence of the HLA-DR16(2) antigen is much more frequent and the presence of HLA-DR13(6) antigen much more rare.

These differences were mainly due to the ethnic variations in HLA phenotypes as well as the specificity and sensitivity of the applied methods. The analysis of the association between particular class II HLA antigens and relative susceptibility or resistance to tuberculosis has been reported rarely and, for futher determination more precise modern molecular techniques, based particularly on the method of the polymerase chain reaction PCR, should be used.

In conclusions: 1) in the patients with pulmonary

tuberculosis in northern Poland, as compared with the control, HLA-DR16(2) antigen was shown to be significantly more frequent. It can be assumed that the presence of this antigen may be connected with a greater risk of pulmonary tuberculosis; 2) in the group of patients, as compared with the control population, the occurrence of HLA-DR13(6) antigen was significantly decreased and the lower relative risk (RR = 0.09) can be connected to its relation with the genes of insusceptibility to tuberculosis.

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