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Association of Human Leukocyte Antigens Class I and II Variants with Susceptibility to Pulmonary Tuberculosis in a Caucasian Population from Northern Spain

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Abstract

Background: Susceptibility to TB seems to be multifactorial, and the development of active disease is probably the result of complex interactions between the host and pathogen, influenced by environmental and genetic factors. The Human Leukocyte Antigen system or HLA seems to be one of these factors. Differences in the distribution of HLA alleles and haplotypes have been found worldwide with conflicting results among different populations and few data concerning Caucasian populations have been reported.

Methods: Distribution of HLA class I and class II alleles was evaluated in 160 Spanish patients with pulmonary tuberculosis, all of them HIV negative, 109 latently infected individuals, and in 262 healthy individuals. All the subjects included in the study belonged to the same geographical area (Cantabria, northern Spain).

Results: HLA-A*02 was found to be significantly more frequent in the latently infected group compared with the control group (34.86% versus 25.19%, *p*=0.009, OR 0.63 IC95% 0.45-0.89) and with patients with pulmonary tuberculosis (34.86% versus 25.79%, *p*=0.03, OR 0.65 IC95% 0.45-0.94). HLA-C*08 was found to be significantly more frequent in pulmonary tuberculosis patients compared with the control group (9.69% versus 5.34%, *p*=0.02, OR 1.91 IC95% 1.12-3.25). HLA-DRB1*04 was found to be significantly more frequent in pulmonary tuberculosis patients compared with the control group (17.19% versus 11.83%, *p*=0.037, OR 1.55 IC95% 1.04-2.29). Finally, HLA-DRB1*07 was found to be significantly more frequent in healthy and latently infected individuals compared with patients with pulmonary tuberculosis (19.66% & 21.10% versus 13.75%, *p*=0.036 & 0.034, OR 0.65 & 0.60 IC95% 0.44-0.96 & 0.38-0.94 respectively). The only haplotype which was significantly more frequent in pulmonary tuberculosis patients versus healthy individuals was the DRB1*04-DQA1*03-DQB1*03 extended three locus haplotype (*p*=0.04, OR 1.53 IC95% 1.03-2.72). All these differences disappeared after statistical correction for multiple comparisons.

Conclusion: Although there were no significant differences in HLA alleles distribution among the three groups after statistical correction, there seems to be a slight trend of certain alleles in conferring protection against or susceptibility to pulmonary tuberculosis, at least in our population.

Keywords: Pulmonary tuberculosis; Spain; HLA class I; HLA class II

Abbreviations: HLA: Human Leukocyte Antigen; TB: Tuberculosis; PTB: Pulmonary Tuberculosis; LTI: Latent Tuberculosis Infection; MTB: *Mycobacterium Tuberculosis*; HWE: Hardy-Weinberg Equilibrium; OR: Odds Ratio; CI: Confidence Interval

Introduction

Tuberculosis (TB) is one of the most important infectious causes of death worldwide. More than 90 million TB patients were reported to the World Health Organization (WHO) between 1980 and 2005, most of them in Asia and sub-Saharan Africa [1]. In Western Europe, Spain has the second highest TB incidence rate after Portugal. In Spain, the estimated pulmonary TB (PTB) incidence rate in 2001 was 16.9 cases per 100,000 per year, the second highest incidence being in Cantabria (northern Spain) with 37.5 cases/100,000/year [2,3].

Although susceptibility to TB is multifactorial, the highly polymorphic HLA genes, besides several other non-HLA genes that seem to be associated with susceptibility to TB [4-10], are strong candidates to act as genetic markers to predisposition or resistance to the development of the disease. HLA class I genes and perhaps more specifically, HLA class II genes, could act directly as disease susceptibility markers [11-14]. In this context, different HLA class II alleles and haplotypes have been associated with susceptibility to TB [4,14,15].

However, there are still few studies on HLA genes and TB in individuals with different ethnic background. This information is particularly scarce for European countries.

Hypothetically, these studies, particularly in genetically well

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defined populations, could offer valuable data: (i) to obtain comparative results between different populations; (ii) to illustrate valuable patterns in designing peptides and, as a consequence, (iii) to provide better therapeutic results when a vaccination approach might be developed.

Furthermore, approximately 90%-95% of individuals infected with *Mycobacterium tuberculosis* (MTB) are able to mount an immune response that halts the progression from latent TB infection (LTI) to active TB disease. This is one of the main reasons that would indicate the need to identify and treat all those with risk factors for TB disease [16,17].

The aim of this study was to determine whether the HLA phenotype is associated with pulmonary TB (PTB) in patients belonging to a genetically well defined population from the North of Spain (Cantabria) where TB, as in other areas of Spain, is an important sanitary problem.

Materials and Methods

Study subjects

Through a retrospective case-control study, we recruited a total of 160 newly diagnosed patients with PTB, and 109 individuals with LTI. As controls, 262 healthy blood donor individuals were randomly selected. Only individuals living in the same area (from all the Cantabrian municipalities) for at least three consecutive generations were included in the study. Furthermore, neither consanguinity nor relationship existed in patients or in healthy controls.

The study was conducted at a 1,200-bed community and teaching hospital. Both, blood donors (mean age, 48 years; range, 18-65 years; male/female ratio, 1.3) LTI (mean age, 52 years; range, 27-63 years; male/female ratio, 1.7), and PTB patients (mean age, 56 years; range, 23-76 years; male/female ratio, 1.5) were of Caucasian background, all of them belonging to the Community of Cantabria (Northern Spain). Detailed description of patients and controls is shown in Table 1. The PTB patients group was selected from patients admitted to the Infectious Unit and the Department of Respiratory Medicine (Hospital Universitario Marqués de Valdecilla) from 2008 to 2012 and who fulfilled clinical, radiological, and bacteriological criteria of active PTB according the standards for the diagnosis and classification of TB developed by the American Thoracic Society and the Centers for Disease Control and Prevention (http://www.cdc.gov/mmwr/). Diagnosis of PTB was made clinically and by X-rays and confirmed by bacteriological (microscopy and culture) procedures. We excluded patients with extrapulmonary TB due to dissemination and subsequent involvement of single or multiple nonpulmonary sites. In the same way, we excluded patients with autoimmune or neoplastic diseases, chronic renal failure, transplant individuals, and patients suffering from alcoholism or drug abuse. Controls had neither previous history of TB nor contact with infected patients. Furthermore, we ruled out the presence of active or latent TB in the control group by performing an interferon-gamma release assay (QuantiFERON TB Gold In-Tube) All subjects were HIV-negative. Procedures used in the study conformed to the principles outlined in the Declaration of Helsinki. All samples were collected with the written consent of the participants. The study protocol was accepted and approved by the Research Ethics Board of the Hospital.

Characteristic	Healthy controls N=262	PTB patients N=162	LTI patients N=109
Gender			
Male	148	97	69
Female	114	65	40
Ratio male/female	1.3	1.5	1.7
Mean age (years)	48	56	52
Range	18-65	23-76	27-63

Table 1: Main demographic data of patients and controls.

The genetic profile (HLA, mtDNA and Y chromosome markers) of this genetically well conserved population has been reported elsewhere [18-21]. Demographic details (age, male/female ratio) were comparable between patients and controls. The procedures conformed to the principles outlined in the Declaration of Helsinki. Local sanitary authorities granted permission for the study; informed consent was obtained and data anonymously recorded. The study protocol was accepted and approved by the Research Ethics Board of the Hospital.

HLA typing

High-molecular-weight genomic DNA was extracted from whole blood as previously described [20]. DNA-based HLA class I and II typing was performed using the Luminex 100 system (Luminex, Austin, TX, USA) and the Lifecodes HLA typing Kits (Gen-Probe Inc., San Diego, CA, USA) following the manufacturer's instructions.

Statistical analysis

Gene and phenotype frequencies were calculated by direct counting. Haplotype frequencies and linkage disequilibrium were analysed by the maximum-likelihood method assuming the Hardy-Weinberg equilibrium law (HWE). Comparison between patients and controls were performed using the χ^2 test or the Fisher's exact test when necessary with Yates' continuity correction. *P* values and odds ratio (OR) with 95% confidence intervals (CI) were calculated using SPSS version 12 (SPSS Inc, Chicago, IL, USA). A value of $p<0.05$ was considered statistically significant.

Bonferroni correction for multiple comparisons was applied in order to avoid false positive results.

Results

The distribution of the HLA class I and II alleles frequencies is shown in Tables 2-4.

Results for HLA-B*, DQA1* and DQB1* are not shown because of the lack of significant differences. All of them were in HWE equilibrium. Regarding HLA class I antigens, HLA-A*02 was found to be significantly more frequent in patients with LTI compared with the control group (34.86% vs 25.19%, $p=0.009$, OR 0.63 IC95% 0.45-0.89) and with PTB patients (34.86% vs 25.79%, $p=0.03$, OR 0.65 IC95% 0.45-0.94) (Table 2). No statistically significant differences were found regarding HLA-B antigens. Finally, HLA-C*08 was found to be significantly more frequent in PTB patients compared with the control group (9.69% vs 5.34%, $p=0.02$, OR 1.91 IC95% 1.12-3.25) (Table 3).

In relation to HLA class II antigens HLA-DRB1*04 was found to be significantly more frequent in PTB patients compared with the control group (17.19% vs 11.83%, $p=0.037$, OR 1.55 IC95% 1.04-2.29) (Table 4). By contrast, HLA-DRB1*07 was found to be significantly more frequent in healthy and individuals with LTI compared with PTB patients (19.66% & 21.10% vs 13.75%, $p=0.036$ & 0.034, OR 0.65 & 0.60 IC95% 0.44-0.96 & 0.38-0.94 respectively). When we applied the Bonferroni correction for multiple comparisons these significant differences disappeared (Table 4).

No significant differences among the three groups were found when we compared all the haplotype frequencies except when we compared between healthy individuals and PTB patients for the DRB1*04;

HLA Allele	Controls n=524	LTI n=218	PTB n=318	Controls vs. LTI			LTI vs. PTB		
HLA-A*	n (%)	n (%)	n (%)	*P*[a]	OR	95%CI	*P*	OR	95%CI
01	56 (10.69)	20 (9.17)	28 (8.81)						
02	132 (25.19)	76 (34.86)	82 (25.79)	0.0098	0.63	0.45-0.89	0.0302	0.65	0.45-0.94
03	69 (13.17)	16 (7.34)	38 (11.95)						
11	43 (8.21)	8 (3.67)	26 (8.18)						
23	19 (3.62)	5 (2.29)	7 (2.20)						
24	40 (7.63)	15 (6.88)	35 (11.01)						
25	12 (2.29)	6 (2.75)	11 (3.46)						
26	16 (3.05)	9 (4.13)	8 (2.52)						
29	48 (9.16)	22 10.09)	30 (9.43)						
30	20 (3.82)	9 (4.13)	10 (3.14)						
31	13 (2.48)	4 (1.83)	9 (2.83)						
32	12 (2.29)	9 (4.13)	8 (2.52)						
33	16 (3.05)	8 (3.67)	14 (4.40)						
34	1 (0.19)	0 (0.0)	2 (0.63)						
66	4 (0.76)	0 (0.0)	0 (0.0)						
68	22 (4.20)	9 (4.13)	10 (3.14)						
74	0 (0.0)	1 (0.46)	0 (0.0)						
80	1 (0.19)	1 (0.46)	0 (0.0)						

[a]Uncorrected *p* value. After Bonferroni correction for multiple comparisons *p* values were not significant.
The sum of the absolute numbers of the two alleles is twice the number of patients and controls. One PTB patient could not be HLA typed and for this reason in all the tables n is 318 instead 320.

Table 2: Distribution of HLA-A* allele frequencies in controls, individuals with LTI and PTB patients.

HLA Allele	Controls n=524	LTI n=218	PTB n=318			
HLA-C*	n (%)	n (%)	n (%)	*P*[a]	OR	95%CI
01	18 (3.44)	8 (3.67)	18 (5.66)			
02	36 (6.87)	11 (5.05)	15 (4.72)			
03	48 (9.16)	18 (8.26)	31 (9.75)			
04	75 (14.31)	29 (13.30)	38 (11.95)			
05	37 (7.06)	16 (7.34)	22 (6.92)			
06	36 (6.87)	15 (6.88)	18 (5.66)			
07	134 (25.57)	49 (22.45)	79 (24.84)			
08	28 (5.34)	13 (5.96)	31 (9.75)	0.022	1.91	1.12-3.25
12	26 (4.96)	14 (6.42)	12 (3.77)			
14	6 (1.14)	7 (3.21)	9 (2.83)			
15	26 (4.96)	16 (7.34)	13 (4.09)			
16	50 (9.54)	21 (9.63)	30 (9.43)			
17	4 (0.76)	1 (0.46)	4 (1.26)			

[a]Uncorrected *p* value. After Bonferroni correction for multiple comparisons *p* values were not significant
No significant differences were obtained when we compared the other groups. One PTB patient could not be HLA typed and for this reason in all the tables n is 318 instead 320.

Table 3: Distribution of HLA-C* allele frequencies in controls, individuals with LTI and PTB patients.

DQA1*03; DQB1*03 extended haplotype (p=0.04, OR 1.53 IC95% 1.03-2.72) (data not shown). In the same way, this difference disappeared after Bonferroni correction.

Discussion

Host genetic factors could explain partly the reason why some individuals would resist TB more successfully than others. However, how much of our genetic burden significantly determines the different responses to this infectious agent is far to be completely understood. Probably, it should be taken in account several other factors such as the existence or not of a concomitant infection, the immunological status of the patient and the genetic variability in the pathogen.

Ethnic differences in susceptibility to TB have been reported [4-10]. Although susceptibility to TB seems to be multifactorial, a relationship between HLA, mainly HLA class II alleles and TB has been defined [4,14,15].

When genetic variations in pathogen polymorphism are superimposed on host genetic heterogeneity, considerable variation may occur in detectable allelic associations [22-24].

Moreover, differences in the HLA allele and haplotype frequencies differ from one geographical area to another. Theoretically, this could be explained as a consequence of evolutionary selection pressures that have allow frequent polymorphism in genes involved in resisting the pathogen contributing to marked differences in allele frequencies at the same loci.

Regarding HLA class I, there are very few reports demonstrating an association of class I alleles with susceptibility or protection against PTB. Our results show an significant association of HLA-A*02 with patientes with LTI compared with healthy subjects or PTB patinets and HLA-C*08 with PTB patients compared with the control group.

Population studies, looking for an association between HLA-A and pulmonary tuberculosis have nor shown convincing results [25]. HLA-A2 has only been found associated with PTB in Egyptian patients. Different HLA-B antigens have been associated with susceptibility to PTB in different populations [25,26].

Balamurugan et al. have demonstrated an association of different HLA-A, B and C alleles with various forms of clinical TB in Indian population [26]. In this work, HLA-A1 antigen was more frequent in patients with PTB. In the same way, the B22, B60 and Cw1, Cw5, Cw6 and Cw7 antigens showed an association with PTB patients. The HLA class I analysis was performed by serological methods. Another work showed an association of HLA-C*01 with susceptibility to PTB in Turkish population [27]. We show, for the first time an association of PTB with HLA-C*08.

HLA Allele	Controls n=524	LTI n=218	PTB n=318	Controls vs. PTB			LTI vs. PTB		
HLA-DRB1*	n (%)	n (%)	n (%)	*P*[a]	OR	95%CI	*P*	OR	95%CI
01	68 (12.98)	23 (10.55)	43 (13.52)						
03	52 (9.92)	19 (8.72)	37 (11.64)						
04	62 (11.83)	31 (14.22)	55 (17.30)	0.0374	1.55	1.04-2.29			
07	103 (19.66)	46 (21.10)	44 (13.84)	0.036	0.65	0.44-0.96	0.034	0.6	0.38-0.94
08	19 (3.63)	15 (6.88)	13 (4.09)						
09	5 (0.95)	2 (0.92)	2 (0.63)						
10	6 (1.14)	3 (1.32)	4 (1.26)						
11	49 (935)	20 (9.17)	31 (9.75)						
12	6 (1.14)	0 (0.0)	4 (1.26)						
13	74 (14.12)	22 10.09)	34 (10.69)						
14	10 (1.91)	8 (3.67)	11 (3.46)						
15	62 (11.83)	26	35 (11.01)						
16	8 (1.52)	3 (1.32)	7 (2.20)						

[a]Uncorrected *p* value. After Bonferroni correction for multiple comparisons *p* values were not significant
No significant differences were obtained when we compared between the other groups. One PTB patient could not be HLA typed and for this reason in all the tables n is 318 instead 320.

Table 4: Distribution of HLA-DRB1* allele frequencies in controls, individuals with LTI and PTB patients.

Association of HLA alleles with PTB has been documented for those countries where TB is a sanitary problem of outstanding importance. In contrast to the HLA class II alleles found associated with susceptibility to PTB in other populations (mainly DRB1*15, DQB1*05 and DQB1*06) [14,28,29], our study revealed a significant association of DRB1*04 with susceptibility to PTB. This weak association between susceptibility to PTB and HLA-DRB1*04 was also found with the three-locus DRB1*04; DQA1*03; DQB1*03 extended haplotype. DRB1*04 alleles seems to be associated with TB in other populations, such as in Chinese Kazakh and in Syrian PTB patients [30,31]. In the same way, DRB1*04; DQB1*03 two locus haplotype has been related to a higher risk of developing TB [32]. Interestingly, DRB1*04 has been found to be a risk factor for tuberculosis among household contacts in families from Brazil, which had at least two cases of tuberculosis diagnosed within the last 5 years [33]. In other report, the HLA-DR4 allele alone or in the presence of the HLA-B14 allele was associated with historical TB patients but not with recently diagnosed patients [34].

All these studies showed the same weak association of this allele or haplotype, and most of them failed because of the lack of statistical correction for multiple comparisons.

On the other hand, when we analysed those alleles related with protection against PTB, we found that DRB1*07 was significantly more frequent in healthy controls and individuals with LTI compared with PTB patients.

Regarding the role of DRB1*07 in TB there are contradictory results. Whereas a meta-analysis performed with different studies totalling 1988 patients and 2897 controls found a reduced risk of developing thoracic TB in those individuals with DR7 antigens [14] two other reports have found an increased risk of PTB among individuals DRB1*07 positives from Iranian and Kirghiz populations [35,36]. No association of the extended DRB1*07; DQA1*02; DQB1*02 because of the high prevalence of the DRB1*07; DQA1*02; DQB1*03 (DQ9) in our population (data not shown).

Due to all these differences in the allele distribution among populations the exact role of the HLA remains unknown. Regarding the role of DRB1*04 allele in conferring susceptibility to PTB, it has been reported that peripheral mononuclear cells from PTB patients and healthy controls stimulated with MTB antigens patients produced higher IL-6 levels [37].

IL-6, together with other cytokines, chemokines, and other important innate immune molecules, have been associated with susceptibility to TB among different populations [38].

To our knowledge, we have reported, for the first time a study regarding the association of HLA class II alleles with susceptibility or resistance to PTB in Spanish patients from Caucasian background. From our data and previous reports we can conclude that candidate HLA variants would be ethnic specific and other genetic and environmental factors could determine resistance or susceptibility to the disease. For the first time, we show a new, although weak association of an HLA class I allele with susceptibility to PTB (HLA-C*08). Further and larger studies in other Spanish populations and in those from other countries are needed to elucidate the true role of HLA class II alleles in the pathogenesis of PTB.

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