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Association of genetic polymorphism of HLA-DRB1 antigens with the susceptibility to lepromatous leprosy

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Abstract. Despite the introduction of multidrug therapy and the overall reduction of leprosy prevalence in Mexico, the disease remains endemic in certain regions of the country. A genetic basis for the immune susceptibility to *Mycobacterium leprae* has already been established in different populations worldwide. In this study, we investigated the possible association of the HLA-DRB1 alleles with leprosy in a Mexican Mestizo population. The results demonstrated that the HLA-DRB1*01 allele is associated with lepromatous and dimorphic leprosy [P<0.001, odds ratio (OR)=4.6, 95% confidence interval (95% CI): 1.8-11.4; and P=0.03, OR=6.2, 95% CI: 1.1-31.6, respectively] and the frequency of the HLA-DRB1*08 allele was found to be significantly lower among leprosy patients compared to controls (P=0.046, OR=2.4, 95% CI: 1-5.8). In conclusion, although the association of the HLA-DR locus with leprosy has been established in different populations and several studies have demonstrated significant differences in the DR alleles, this study demonstrated an association of the HLA-DRB1*01 allele with susceptibility to lepromatous and dimorphic leprosy, as well as an association of the HLA-DRB1*08 allele with protection against leprosy in a Mexican Mestizo population.

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Introduction

Leprosy is a chronic infectious disease caused by the intracellular acid-fast *Mycobacterium leprae* (*M. leprae*) bacilli. It affects the skin and peripheral nerves of susceptible individuals, causing irreversible impairment of nerve function and consequent chronic disability (1). As a result of the use of multidrug therapy, promoted by the World Health Organization, the global prevalence of this disease has been significantly reduced (2,3). However, leprosy remains a public health problem (4), affecting ~500,000 individuals annually worldwide (3). In Mexico, 215 new cases of leprosy were reported until the end of the first quarter of 2012, resulting in an estimated prevalence of 0.480 cases per 100,000 inhabitants, with a predominance of males over females. In addition, the proportion of multibacillary cases has been found to increase in several Mexican populations, concomitantly with the declining incidence (2,5). However, a previous epidemiological study demonstrated that only a small percentage of individuals exposed to the bacillus develop the disease, suggesting that the majority of individuals are immunologically competent to reject infection by developing effective cellular immune responses (6).

The clinical spectrum of leprosy includes two poles, the tuberculoid (TT) or paucibacillary (PB) and the lepromatous (LL) or multibacillary (MB) poles, with several intermediate (I) or borderline forms [borderline tuberculoid (BT), borderline borderline (BB) and borderline lepromatous (BL)]. Immunologically, the LL pole is characterized by a Th2 T-cell immune response, antibody complex formation, absence of granulomas and failure to restrain *M. leprae* growth. By contrast, the TT pole presents with a Th1 T-cell cytokine response, a vigorous T-cell response

to *M. leprae* antigens and containment of the infection in well-formed granulomas (7).

Accumulated evidence currently indicates that exposure to *M. leprae* is necessary but not sufficient to explain the susceptibility to leprosy and previous studies demonstrated that the pathogenesis of leprosy is a two-step process, in which a group of genes controls susceptibility to infection *per se*, whereas a different group of genes controls the clinical manifestation of the disease (1,8). Evidence that host genes affect susceptibility to leprosy or its various clinical forms is supported by data from a wide variety of sources, including twin studies, segregation analyses, family-based linkage and association studies, candidate gene association studies and, most recently, genome-wide association studies (GWASs) (7,9). One recent genome-wide scan pinpointed chromosome 6p21 as a leprosy susceptibility locus (10). This region harbors the human leukocyte antigens (HLA) gene cluster, which has been extensively investigated for its role in the pathogenesis of leprosy. Previous candidate gene studies detected an association of leprosy with class I and II genes. However, class II genes, particularly the DRB1 locus, were found to be more consistently associated with leprosy (6,11).

Several studies reported an association of the HLA-DR locus with susceptibility or resistance to leprosy in different populations, demonstrating the significance of this locus in the pathogenesis of leprosy and highlighting the fact that certain HLA-DR alleles are associated with susceptibility to leprosy *per se*, whereas other alleles are associated with predisposition towards a particular clinical spectrum of this disease (12). In this context, independent replication studies in several ethnically different populations are required for multifactorial diseases, such as leprosy (6). Considering these facts and taking into consideration that the genetic susceptibility to leprosy in the Mexican Mestizo population has received little attention, the aim of this study was to investigate the association of HLA-DRB1 alleles with leprosy in a Mexican Mestizo population.

Subjects and methods

Subjects. In this study, we recruited a total of 52 patients with leprosy (39% female and 61% male), with a mean age of 65±14 years (range, 18-86 years), who were classified according to the international criteria establish by Ridley and Jopling (13). The patients were residents from the states of Sinaloa (48%), Jalisco (27%), Guanajuato (11%), Oaxaca (6%), Veracruz (2%), Nuevo Leon (2%), Guerrero (2%) and Hidalgo (2%). Forty-one patients were classified as LL, 2 as TT, 6 as dimorphic (D) and 3 as I. All leprosy cases were MB, with the exception of 5 PB (i.e., 2 TT and 3 I). A total of 99 healthy individuals (50% female and 50% male), with a mean age of 40±10 years (range, 28-52 years), unrelated to the patients and matched by ethnicity, were included as the control group. Ethnically, the patients and the controls were classified as Mestizos, who are defined as individuals born in Mexico, with a Spanish-derived last name and Mexican ancestors at least back to the third generation. Mestizos are the result of 500 years of admixture between Spaniards, Amerindians and Africans and they currently represent the majority of the Mexican population (>90%) (14,15).

A written informed consent was obtained from all the subjects prior to their enrollment in this study, according to the Helsinki Declaration.

HLA-DRB1 typing. Genomic DNA from all the subjects included in this study was purified from peripheral blood leukocytes, according to the method described by Miller (16). Blood was collected by single peripheral venepuncture, according to guidelines approved by the Internal Review Boards of the participant hospitals. The HLA-DRB1 locus was genotyped based on the hybridization of labeled single-stranded polymerase chain reaction products to sequence-specific oligonucleotides, using the LifeCodes HLA-DRB1 Typing kit for use with Luminex (Gen-Probe Transplant Diagnostics, Inc., Stamford, CT, USA) following the manufacturer's recommendations. Data were analyzed using Quicktype for Lifecodes version 3.0 software to determine the HLA alleles.

Statistical analysis. The allele frequencies were calculated by direct counting and the differences in the distribution of the alleles between patients and controls were analyzed using the χ^2 test or the Fisher's exact test. Statistically significant P-values (≤0.05) were corrected taking into consideration the number of alleles observed (pc) (17). The strength of the associations was estimated by calculating the odds ratio (OR). Statistical analyses were performed using Arlequin software, version 3.5.1.2 (Swiss National Science Foundation, Bern, Switzerland).

Results

Subjects and allele frequency. A total of 151 Mexican Mestizo individuals (52 patients with leprosy and 99 healthy controls) were genotyped for the HLA-DRB1 locus. Table I summarizes the allelic frequency distributions of the HLA-DRB1 alleles in leprosy patients and controls. Of the 13 HLA-DRB1 alleles determined (*01, *03, *04, *07, *08, *11, *13, *14 and *15 being the more frequent) we only identified 2 alleles exhibiting a statistically significant difference in frequency between patients and controls. The frequency of the HLA-DRB1*01 allele among leprosy patients was significantly higher compared to that observed among healthy controls [P<0.001, OR=5.6, 95% confidence interval (95% CI): 2.4-13.3]. By contrast, the frequency of the HLA-DRB1*08 allele was significantly lower among leprosy patients compared to that among controls (P=0.046, OR=2.4, 95% CI: 1-5.8) (Table I).

Association of alleles with leprosy subtypes. We investigated the possible association of the HLA-DRB1 alleles with the clinical subtypes of leprosy. For this purpose, taking into consideration that the majority of cases of leprosy in Mexico are MB and due to the fact that only 5 samples were collected from patients with the PB form, we only considered the MB form and healthy controls for this analysis. We observed that the frequency of the HLA-DRB1*01 allele was significantly higher in the MB group compared to that among controls (P<0.001, OR=4.5, 95% CI: 1.84-11.4) (Table II). This association was confirmed when we performed the analysis in the groups

Table I. Frequencies of HLA-DRB1 alleles in patients with leprosy and healthy controls.

HLA-DR alleles	Leprosy (N=52)		Controls (N=99)				
	n	gf	n	gf	pc	OR	95% CI
01	24	0.230	10	0.050	<0.001	5.6	2.4-13.3
04	20	0.192	47	0.237	0.45	-	-
07	18	0.173	22	0.111	0.18	-	-
08	8	0.077	33	0.167	0.046[a]	2.4	1-5.8
13	8	0.077	10	0.050	0.50	-	-
14	6	0.057	21	0.106	0.23	-	-
15	6	0.057	13	0.065	0.98	-	-
03	6	0.057	11	0.055	0.93	-	-
11	4	0.040	20	0.101	0.092	-	-
10	2	0.019	1	0.005	0.273	-	-
09	1	0.009	3	0.015	1	-	-
12	0	0	2	0.010	0.546	-	-
16	1	0.009	5	0.025	0.668	-	-

N, number of individuals; gf, genetic frequencies; n, number of alleles; pc, Yates corrected P-values corrected taking into consideration the number of alleles observed; OR, odds ratio; 95% CI, 95% confidence interval. [a]Inverted value of OR.

Table II. Frequencies of HLA-DRB1 alleles in multibacillary (MB) patients and healthy controls.

HLA-DR alleles	MB (N=41)		Controls (N=99)		MB vs. controls		
	n	gf	n	gf	pc	OR	95% CI
01	16	0.195	10	0.050	<0.001	4.5	1.84-11.4
04	16	0.195	47	0.237	0.53	-	-
07	14	0.171	22	0.111	0.24	-	-
08	8	0.098	33	0.166	0.19	-	-
13	7	0.085	10	0.050	0.40	-	-
14	5	0.061	21	0.106	0.33	-	-
15	4	0.049	13	0.065	0.79	-	-
11	4	0.049	20	0.101	0.23	-	-
03	3	0.037	11	0.055	0.71	-	-
09	1	0.012	3	0.015	0.71	-	-
10	2	0.02	1	0.005	0.42	-	-
16	1	0.012	5	0.025	0.81	-	-
12	1	0.012	2	0.010	0.89	-	-

N, number of individuals; n, number of alleles; gf, genetic frequencies; pc, Yates corrected P-values taking into consideration the number of alleles observed; OR, odds ratio; 95% CI, 95% confidence interval.

based on clinical classification, where we observed a strong association of the HLA-DRB1*01 allele with LL and D leprosy (P<0.001, OR=4.6, 95% CI: 1.8-11.4; and P=0.03, OR=6.2, 95% CI: 1.1-31.6, respectively) (Table III). Furthermore, as regards the HLA-DRB1*07 allele, we observed that there was a statistical tendency to D leprosy, although the association was not statistically significant (P=0.069). In the case of the other HLA-DRB1 alleles, no statistically significant differences were observed between patients and controls (P≥0.05) (Table III).

Discussion

It has been established that certain individuals who are exposed to the *M. leprae* bacillus are resistant to disease and raise an effective immune response, whereas others will develop clinical disease (18), suggesting that the development of leprosy is significantly affected by host genetics factors. An observation supporting the significant role of genetic factors in the development of leprosy is that several genes and genomic regions have been implicated in the

Table III. Frequencies of HLA-DRB1 alleles in leprosy subgroups and healthy controls.

HLA-DR alleles	LL (N=41)					D (N=6)					Controls (N=99)	
	n	gf	pc[a]	OR	95% CI	n	gf	pc[b]	OR	95% CI	n	gf
01	16	0.195	<0.001	4.6	1.8-11.4	3	0.250	0.03	6.2	1.1-31.6	10	0.050
03	5	0.061	0.91	-	-	0	0	0.86	-	-	11	0.055
04	16	0.195	0.53	-	-	2	0.167	0.83	-	-	47	0.237
07	14	0.171	0.24	-	-	4	0.333	0.069	4	0.9-16.2	22	0.111
09	1	0.012	0.31	-	-	0	0	0.41	-	-	3	0.015
10	2	0.02	0.42	-	-	0	0	0.05	-	-	1	0.005
12	0	0	0.89	-	-	0	0	0.23	-	-	2	0.010
08	8	0.098	0.19	-	-	0	0	0.25	-	-	33	0.167
13	6	0.073	0.64	-	-	2	0.167	0.29	-	-	10	0.050
14	5	0.061	0.33	-	-	1	0.083	0.81	-	-	21	0.106
15	4	0.049	0.79	-	-	0	0	0.76	-	-	13	0.065
11	4	0.049	0.23	-	-	0	0	0.51	-	-	20	0.101
16	1	0.012	0.75	-	-	0	0	0.67	-	-	5	0.025

LL, lepromatous leprosy; N, number of individuals; D, dimorphic leprosy; n, number of alleles; gf, genetic frequencies; pc, Yates corrected P-values taking into consideration the number of alleles observed; OR, odds ratio; 95% CI, 95% confidence intervals. [a]LL vs. controls; [b]D vs. controls.

complex genetic mechanisms controlling host susceptibility to disease (1).

Leprosy is one of the first human diseases in which human leukocyte antigen genes were demonstrated to co-determine disease outcome (18). These associations with HLA molecules may be the major genetic determinants of the disease phenotype, which is consistent with the large numbers of available studies that demonstrated an association between the DR alleles and the different clinical subtypes of leprosy, or even with leprosy *per se* (19).

It was previously demonstrated that, in Mexicans, the HLA-DRB1*03 allele is associated with the TT clinical subgroup (20) and a previous family study demonstrated an association of the HLA-DRB1*1501 allele with LL leprosy (21). In this study, we reported the association of the HLA-DRB1*01 allele with susceptibility to LL and D leprosy and we observed that the HLA-DRB1*08 allele was associated with protection against leprosy. Of note, the HLA-DRB1*01 allele appears to have been introduced in this population through admixture with Caucasians, whereas the HLA-DRB1*08 allele is the second most common allele encountered in the normal Mexican population and it is present in Mestizos, as well as in autochthonous populations from Mexico, which may be associated with natural selection from infectious diseases following contact with Europeans during the 16th century (22). These data confirm the role of HLA in the genetic susceptibility to the development of leprosy in several populations worldwide and also confirm the role of HLA-DR in the clinical outcome of this disease.

As regards other endemic countries, such as Vietnam and Brazil, the HLA-DRB1*10 allele has been associated with susceptibility to leprosy, whereas the HLA-DRB1*04 allele has been associated with protection against leprosy (6). A previous study from Brazil demonstrated that the HLA-DRB1*09 allele was associated with susceptibility to and the HLA-DRB1*04 allele with protection against BL (23). In Argentina, the HLADRB1*04 allele was found to be associated with protection against MB and the HLA-DRB1*0402 allele with protection against leprosy *per se* (24).

In a Chinese population, the HLA-DRB1*09 allele was associated with the development of indeterminate leprosy only in young people, whereas the HLA-DRB1*15 allele was associated with susceptibility to leprosy *per se* (12). The HLA-DRB1*1501, HLA-DRB1*1502 and HLADR-B1*1404 alleles were associated with an increased susceptibility to TT in an Indian population (25). A study from Indonesia demonstrated that the HLA-DRB1*02 allele confers susceptibility to, whereas the HLA-DRB1*12 allele is associated with protection against leprosy (26). In Egypt, HLA-DR2 was associated with susceptibility to leprosy *per se* (27) and in northern India, the HLA-DRB1*15 allele (a HLA-DR2 subtype) was associated with MB (28).

Considering those results, to the best of our knowledge, this study was the first to report evidence of an association between the HLA-DRB1*01 allele and susceptibility to LL and between the HLA-DRB1*08 and protection against leprosy *per se* in a Mexican Mestizo population, which possesses a high degree of genetic heterogeneity. HLA type studies demonstrated that, in general, Mexican Mestizos harbour several more Amerindian genes compared to European and African haplotypes (29).

In conclusion, our study described the genotyping of the HLA-DRB1 locus in Mexican Mestizos diagnosed with leprosy and healthy individuals. Our findings suggest that the HLA-DRB1*01 and HLA-DRB1*08 alleles may be novel genetic markers for susceptibility to and protection against leprosy, respectively, in a Mexican Mestizo population. The

investigation of leprosy in different endemic regions may provide more insight into the pathogenesis of this disease and its heterogeneous distribution in Mexico.

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