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Immunogenetics of mixed connective tissue disease in a Mexican Mestizo population

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ABSTRACT

Objective

The aim of this study was to determine the HLA antigens in Mexican Mestizo patients with mixed connective tissue disease (MCTD).

Methods

We studied 30 patients with MCTD and 99 healthy controls. HLA-A, -B, and -DQ antigens were typed by microlymphocytotoxicity assays. DRB1, DQA1 and DQB1 alleles were oligotyped.

Results

HLA-A2 and HLA-B35 were the most frequent MHC class I alleles in MCTD patients, although they were not statistically more frequent than in the controls. According to serological tests, the most frequent DQ allele in the patients was DQ1, which was statistically increased when compared with controls (p = 0.0051). By oligotyping, the DR1 allele and the DQB1 0501 specificities were significantly increased in the patients vs. controls (p = 0.032 and 0.027, respectively).*

Conclusion

The elevated levels of DQ1 found in Mexican MCTD patients, although weak, may indicate a particular genetic susceptibility, since there are previous reports of associations of other alleles (such as DR4) with MCTD in other populations. The increase in DQB1 0501 may account for the increase in DQ1. DQB1*0501 has also been reported in black patients with anti-RNP autoantibodies, compared with black patients without anti-RNP or anti-Sm.*

Introduction

Mixed connective tissue disease (MCTD) is an autoimmune disorder characterized by the presence of high titers of serum autoantibodies against small nuclear ribonucleoproteins (U-snRNPs) (1), in particular against the U1 polypeptide of 70 kD. It has been suggested that MCTD represents a distinct clinical entity (2). Patients with MCTD have a core set of clinical manifestations that separate them from patients with other connective tissue diseases, such as systemic lupus erythematosus (SLE), polymyositis/dermatomyositis (PM/DM), scleroderma (Scl), rheumatoid arthritis (RA), and Sjögren's syndrome (SS).

Several authors have proposed clinical and laboratory criteria based on the small group of clinical manifestations that frequently occur in MCTD patients (3-5). Among these manifestations are edema of the hands, Raynaud's phenomenon, myositis, polyarthritis, and sclerodactyly. Alarcón-Segovia and Cardiel compared three sets of criteria for the classification of MCTD, and found them to correlate well (6). Nevertheless, some authors consider that the classification scheme for MCTD remains unclear (7-10).

Various associations of HLA antigens with MCTD have been reported. Thus, Sasazuki *et al*. described an association of HLA-B7 and HLA-Dw1 (11). In an English study DR4 was found to be significantly increased in MCTD (12), particularly in patients with arthritis, and the Gm allotype frequencies in MCTD patients were different compared with controls. Nishikai and Sekiguchi reported an association of DQw3 and anti-RNP antibodies in a group of patients with different connective tissue diseases, including MCTD and SLE (13).

MCTD patients with increased IgG autoantibodies against the U1-70 kD polypeptide have an increased prevalence of DR4 antigen compared with controls (14). Furthermore, molecular biology studies have shown that most MCTD patients with DR4 or DR2 alleles have a region of homology consisting of seven amino acids in the DRB1 gene (15). This "shared epitope" of DR molecules in MCTD may be important for the modulation of the autoimmune response to the U1-70 kD antigen (16).

Some authors have proposed that MCTD may actually be a subset of SLE (17), and others that MCTD can evolve into a more defined connective tissue disease (10). Still others, however, have found that from an immunogenetic point of view MCTD is distinct from SLE (18).

The normal Mexican Mestizo population is characterized by a particular HLA profile. The most frequent alleles in this population are A2, B16, DR4, and DQw3

(19, 20). The frequencies of these alleles distinguish the Mexican Mestizos from Caucasian populations. With regard to the rheumatic diseases, the Mexican population also displays a unique pattern of susceptibility compared with other ethnic groups. In Mexican RA patients, there is a significant increase of A1, DR3 and DQ2, and a significant decrease of A31 when compared with controls (21); in Scl patients, the frequencies of DR5 and DRw52 are increased (22); and in SLE patients the frequencies of DR3 and DR7 are increased (23). Since it is well established that there are differences in HLA frequencies among different ethnic groups, both in health and disease, the determination of HLA allele frequencies in other autoimmune diseases in Mexicans would appear to be useful.
In the present study, we determined the HLA-DR and HLA-DQ antigens in a group of 30 Mexican Mestizo patients with MCTD.

Materials and methods

Study subjects
We initially recruited 52 patients with a probable diagnosis of MCTD. These patients were followed in the Department of Immunology and Rheumatology of the Instituto Nacional de la Nutrición Salvador Zubirán, a referral center in Mexico City. MCTD was confirmed in 30 of these 52 patients. All cases were classified by a certified rheumatologist, according to the criteria proposed by Alarcón-Segovia and Villarreal (3). This classification was blind to the HLA results. The control group consisted of 99 healthy subjects.

Serological typing and oligotyping
Serological typing was performed in the 30 MCTD patients. MHC class I antigens (HLA-A and -B) were determined by microlymphocytotoxicity assays (24) with peripheral blood mononuclear cells. HLA-DQ antigens were also determined by microlymphocytotoxicity assays with isolated peripheral blood B cells. Genomic DNA from peripheral blood mononuclear cells of 26 MCTD patients (52 haplotypes) was amplified by the polymerase chain reaction (PCR) of polymorphic gene segments of HLA-DRB1 and of polymorphic gene segments of the second exon of HLA-DQA1 or DQB1, using primers of the Twelfth International HLA Workshop, and the DRB1, DQA1 and DQB1 alleles were typed by dot-blot hybridization with sequence specific oligonucleotides.

Ethics
The procedures followed were in accordance with the standards of the Ethics Committee of the Instituto Nacional de la Nutrición Salvador Zubirán.

Statistics
HLA gene frequencies were calculated by direct counting. Statistical comparison of the frequencies was performed by the χ^2 and Fisher's exact tests, using the statistical program EPIINFO. A p value lower than 0.05 was considered as statistically significant.

Results

The mean age of the 30 patients with MCTD was 42 years (SD = 12.04; 21 - 67 years). Twenty-seven patients were female and three were male. The patients had been followed for an average of 13.27 years (SD = 7.83; 3 - 29 years). The median disease duration before diagnosis was 6 years (1 - 24 years). We consider that our definitive diagnosis of MCTD in the 30 patients was supported by the careful follow-up that was carried out in each case. Table I shows the number and frequency of patients who fulfilled each of the criteria for the classification of MCTD.

Serological typing
There were no significant differences in the HLA-A and -B allele frequencies between the 30 MCTD patients and the controls (data not shown). In the patients, the most frequent alleles in these loci were increased when compared with controls. These alleles were HLA-A2 (0.70 vs. 0.48 in the controls) and HLA-B35 (0.48 vs. 0.25 in the controls).
In the HLA-DQ locus, there was a significant increase of DQ1 in MCTD patients when compared with the controls (0.71 vs. 0.38, p = 0.0051, odds ratio = 4.08; 95% confidence interval: 1.34 - 13.82). The DQ2 and DQ3 alleles were decreased and the DQ4 allele was increased in the patients, but these differences were not statistically significant.

Table I. Number of patients who fulfilled the criteria for the classification of mixed connective tissue disease (MCTD) (ref. 3).

Criteria for the classification of MCTD	Patients No.	Freq.
ENA ≥ 1:1600	30	(1.0)
Acrosclerosis	22	(0.73)
Myositis	19	(0.63)
Synovitis	28	(0.93)
Edema of the hands	26	(0.87)
Raynaud's phenomenon	29	(0.97)

ENA: extractable nuclear antigen.

DRB1, DQA1 and DQB1 oligotyping
Table II shows the gene frequencies of class II MHC alleles determined by oligotyping. In the HLA-DR locus, the most common allele was DR4 (0.326), but its frequency was not significantly different from that of the controls (0.237). On the other hand, the DR1 allele was significantly increased in the patients compared with controls (0.134 vs. 0.050, p = 0.032, odds ratio = 2.92, 95% confidence interval: 0.89 - 9.01).
Since the DQ1 allele was found to be significantly increased in the patients when determined by serological tests, it was decided to study its associated DQB1 specificities. It was found that the DQB1*0501 specificity associated with DQ1 was significantly increased in MCTD patients compared with controls (0.153 vs. 0.060, p = 0.027, odds ratio = 2.82, 95% confidence interval: 0.93 - 7.99).

Discussion

The present study analyzed the HLA alleles in 30 Mexican MCTD patients. Because of the controversy that still exists regarding the exact definition of MCTD, a strict and careful classification of the patients was carried out.
In the serological tests, the only HLA allele that was significantly increased in the patients with respect to the normal controls was DQ1. To our knowledge, this difference has not been reported before. In contrast to previous reports (12, 14, 18), we did not find a significant increase in DR4 in our patients. This could

Table II. Gene frequencies of class II MHC alleles determined by oligotyping in Mexican patients with MCTD and in healthy controls.

	Patients (n = 52 haplotypes)		Controls (n = 198 haplotypes)		
	n	gf*	n	gf*	p
HLA-DR					
DR1	7	0.134	10	0.050	0.032¶
DR2	6	0.115	18	0.090	0.593
DR3	3	0.057	11	0.055	0.952
DR4	17	0.326	47	0.237	0.187
DR5	2	0.038	22	0.111	0.113
DR6	3	0.057	31	0.156	0.064
DR7	4	0.076	20	0.101	0.599
DR8	8	0.153	33	0.166	0.824
DR9	1	0.019	3	0.015	0.834
DR10	1	0.019	1	0.005	0.307
HLA-DQA1					
0101	8	0.153	20	0.101	0.282
0102	6	0.115	17	0.085	0.512
0201	4	0.076	22	0.111	0.472
0301	18	0.346	51	0.257	0.203
0401	8	0.153	33	0.166	0.824
0501	8	0.153	45	0.227	0.248
HLA-DQB1					
0201	7	0.134	33	0.166	0.547
0301	5	0.096	35	0.176	0.158
0302	17	0.326	47	0.237	0.187
0303	1	0.019	0	0.000	0.050
0402	8	0.153	33	0.166	0.824
0501	8	0.153	12	0.060	0.027¶¶
0602	6	0.115	15	0.075	0.359

*gf = gene frequency;
¶ Fisher's exact test p value = 0.039, odds ratio = 2.92, 95% confidence interval: 0.89 - 9.01;
¶¶ Fisher's exact test p value = 0.033, odds ratio = 2.82, 95% confidence interval: 0.93 - 7.99.

indicate that the differences in genetic susceptibility may be the result of differences in ethnic background or environmental factors.

The significant increase in DQB1*0501 specificity in our patients could account for the elevated DQ1 frequency. Interestingly, it has been reported that black patients with anti-RNP antibodies have significantly higher frequencies of DQA 1*0101 and DQB1*0501 compared with black patients without anti-RNP or Sm (25). The same authors also found that white patients with anti-RNP showed an increase of DQB1*0302 when compared with healthy controls, and a rise of DQ A1*0101 and DQB1*0501 when compared with patients without anti-RNP or Sm. In the present study, the specificities of DQB1*0302 and DQA1* 0101 were increased in the MCTD patients compared with controls, although not significantly.

Our results could thus confirm an association of DQB1*0501, and possibly of DQA1*0101 and DQB1*0302, with anti-RNP antibodies, although the study sample was too small for definitive conclusions to be drawn.

It is worth mentioning that the relationship between HLA and disease can be a strong, medium or weak association, combined with environmental factors. A strong association has been described worldwide between the B27 allele and ankylosing spondylitis (26), and a less definite or medium association has been reported between DR4 and rheumatoid arthritis (27). In the present study the association of DR1 and DQB1*0501 with MCTD may represent a weak association, in which other genetic or environmental factors could also contribute to the susceptibility. Thus, the association of the HLA genes and different diseases constitutes a continuum, where strong, medium or weak values can be observed depending upon the influence of other genes and/or environmental factors.

The etiopathogenicity of autoimmune diseases may therefore be understood in the context of the varying contributions of genetic and non-genetic factors to the disease susceptibility (28). In the case of MCTD, the influence of the HLA alleles is not as strong as that described for ankylosing spondylitis, SLE, or insulin-dependent diabetes mellitus (IDDM) (29).

When defining MCTD as an independent entity, it should be remembered that its distinction from other rheumatic diseases is based mainly upon its clinical characteristics. The weak association that we found with the HLA alleles does not define MCTD, because their contribution is small. Nevertheless, an association with certain HLA alleles does seem to exist, although it is not universal and could play a role only in the context of other genetic and environmental factors. In this respect, the stronger association of the B8 DR3 haplotype with SLE (23) alone is not sufficient to define this autoimmune disease. This same haplotype is also associated with other diseases, such as Scl and autoimmune thyroiditis (30). Again, it can be said that certain HLA genes may increase disease susceptibility, and that environmental factors, including bacteria or viruses, may be added to result in a definite entity.

This work presents the immunogenetic analysis of a group of carefully classified Mexican patients with MCTD. The association of DQ1 and its DQB1*0501 specificity with MCTD, although weak, is distinct from the assocations of HLA alleles with SLE, RA, and Scl. We had previously found that in Mexican patients SLE is associated with DR3 and DR7 (23); RA with A1, DR3 and DQ2 (21); and Scl with DR5 and DRw52 (22). Immunogenetic distinctions should always be considered in the context of the clinical differences between these autoimmune diseases.

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