Immunogenetics and clinical aspects of Takayasu's arteritis patients in a Mexican Mestizo population

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Abstract

Objective

The aim of the present work was to study the association between HLA alleles and Takayasu's arteritis in Mexican Mestizo patients.

Methods

The study included 26 Mexican Mestizo patients with Takayasu's arteritis and 99 healthy unrelated individuals. HLA-A, -B and -DR alleles were determined by polymerase chain reaction PCR-SSP.

Results

Increased gene frequencies were demonstrated for HLA-B15 (p=0.009, pC=0.020, OR=3.24, EF=11.9%) and HLA-B52 (p=0.008, pC=0.027, OR=5.16, EF=7.7%), and a decreased frequency for the HLA-A24 allele in patients compared to normal controls (p=0.035, pC=NS, PF=11.1%). When HLA typing was correlated to clinical features in 24 cases, we found an increased frequencies of HLA-DR14 in patients with systemic arterial hypertension (p=0.005, pC=0.004, OR=24.6, EF=38.3%) and HLA-A2 on patients with pulmonary involvement (p=0.034, pC=0.036, OR=3.67, EF=40.4%) when compared to patients without these clinical manifestations.

Conclusion

These data confirm HLA-B52 as a relevant susceptibility allele for Takayasu's arteritis and suggest that HLA-B15 could be important as a marker of the disease in Mexican patients. Other class I and/or class II alleles could also be relevant as markers for the clinical features present in these patients.

Key words

Major histocompatibility complex (MHC), HLA antigens, Takayasu's arteritis, Mexican Mestizo population.

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Introduction

Takayasu's arteritis (TA) is a chronic large vessel vasculitis, which involves elastic arteries such as the aorta and its main branches, and the pulmonary artery to a lesser extent (1). Reports of aorto-arteritis can be traced to the 18th and 19th centuries; in 1908 this disease was recognized by Mikito Takayasu, an ophthalmologist, because a peculiar eye fundii appearance (2-4). Although TA is common in Asia it has a worldwide distribution, predominates in non-Caucasian young females, and clinical findings usually reflect ischaemia and multi-organ involvement:cardiac, cutaneous, renal, eye and central nervous system, as well as pulmonary, muscle-skeletal and gastrointestinal findings. Inflammatory features are recognized in some cases and arterial inflammation may cause stenosis, occlusion and occasionally aneurysms (5-10). The diagnosis of TA is always a challenge to clinicians. Absent peripheral pulses and blood pressure differences are cardinal findings. These, together with "pulselessness" due to arterial obstructions in reproductive-aged women, are considered to be classification criteria by the American College of Rheumatolgy (11) and important clues for diagnosis by Sharma (12), although the diagnosis must necessarily be confirmed by total aortography demonstrating the characteristic appearance of the diseased aorta (13). Panaortogram and other imaging studies are useful to evaluate the extent of the disease and monitor progression and therapy (14).

The etiology of TA remains unknown; an immune pathogenesis has been suspected for many years but has not yet been proven (15, 16).

Ethnicity, familial aggregation and the association in some cases with immune-mediated disease have prompted studies on immunogenetic markers (17, 18). Since 1972 HLA analysis of TA has been studied in Japan to search for genetic factors. Some of the Major Histocompatibility Complex (MHC) genes located in the short arm of the human sixth chromosome (6p21.3) have been associated to genetic susceptibility to TA. Associations of HLA antigens with TA have been reported, mostly on Japanese populations. Dong *et al*. detected an increased B52 allele frequency that was present in 53.1% of the 64 patients and in 24.0% of the 317 studied controls ($p < 0.00001$; relative risk= 3.59). Also, a positive association between HLA and TA was observed with DRB1*1502 allele (46.9% vs 24.0%; $p<0.0003$; relative risk= 2.80) (19). A study on Korean TA patients reported a statistically significant association of HLA-B52 (19% vs 6%; $p<0.02$, relative risk = 3.59) in a sample of 141 patients (20). In Thailand HLA-A31 and HLA-B52 have been associated with TA ($p<0.05$) (21). Among Indian patients an association with HLA-B5 has been reported (22). This data was recently corroborated in 104 unrelated Indian patients who presented an increased frequency of HLA-B5 ($p<1 \times 10^{-6}$, relative risk= 3.08) (23). The presence of HLA-A2, -A9, -BW35 and DR7 was observed in four cases of TA in female Arabs (24). In previous North American studies, a HLA-DR4/MB3 (DQw3) (25) association was found and a HLA-DR1-TA negative association was reported later in 21 patients (0% in patients vs 23.0% in healthy controls, $p=0.02$), suggesting that this antigen might be protective against the development of this disease (26).

Previous studies in Mexico show that the genetic structure of the Mexican Mestizo population has 56% Amerindian genes, 40% Caucasian genes and 4% Negroid genes (27), TA is a common condition in this population and it has been associated, in small preliminary studies (12 patients), mainly with HLA-DR6 ($pC=0.0007$, relative risk = 5.08) (28). In this work we applied molecular studies to a cohort of the Mexican Mestizo population who had a definitive diagnosis of TA in order to determine which HLA alleles are involved in the susceptibility to this disease.

Materials and methods

Patients

We studied 26 consecutive Mexican Mestizo patients over a period of 5 years who attended the Immunology Clinic at the Instituto Nacional de Car-

diología "Ignacio Chávez" in Mexico City. Twenty-four of them were women and 2 were men, ranging in age from 17 to 73 years (median 38.5). The TA diagnosis was based on clinical features and characteristic aortography, which was classified according to Numano (29) as follows: type I (involving the branches of aortic arch); type IIa (ascending aorta, aortic arch and its branches); type IIb (type IIa + thoracic descending aorta); type III (descending thoracic and abdominal aorta); type IV (abdominal aorta and/or renal arteries) and type V (aortic arch, thoracic and abdominal aorta). All patients fulfilled the 1990 American College of Rheumatology Criteria for the Classification of Takayasu's Arteritis (18). Clinical characteristics were evaluated in 24 patients who agreed to participate. After clinical data were obtained, we searched for HLA allele differences and specific organ damage. This study was approved by our Institute's review committee and all subjects gave their informed consent.

Controls

Our normal controls included 99 healthy unrelated Mexican Mestizo individuals (79 women and 20 men). Inclusion criteria included Mexican ancestry up to the grandparents and no known HLA-associated disease. Control individuals live in Mexico City and should be considered as representative for most of Mexican population, since Mexico City has been an important immigration region, increasing from 5 million to 20 million people during the past ten years.

HLA typing

Venous blood samples were obtained from patients and controls. Genomic DNA was obtained by the Salting Out technique (30). Genetic variants of HLA-A, -B and -DR were determined by PCR-SSP (polymerase chain reaction with sequence specific primers) (Pel. Freez, Clinical Systems, Brown Deer, Wisconsin, USA) and electrophoresis in 2% agarose gel with ethidium bromide. The interpretation of the results was carried out using the computer program developed by the manufacturer for this purpose (Clinical Systems).

Statistical analysis

Mantel-Haenzel ^{2} analysis was performed to determine the significance of differences between the two groups. This was combined with 2 X 2 contingency tables using EPIINFO statistical software (Version 5.0; USD Incorporated 1990, Stone Mountain, Georgia). Fisher's exact test was used if the number of any cells was less than 5. P values were corrected according to Yates (31) and were considered statistically significant if < 0.05. The strength of an association was estimated by the relative risk and etiologic fraction (EF) (32). Relative Risk with 95% confidence intervals (CI) was calculated as the odd ratios, (OR) according to Woolf's method (33).

Results

Clinical features

Twenty-four of the patients were women and 2 were men, ranging in age from 17 to 73 years (median 38.5); the age at disease onset was between 9 and 42 years (median 21). In all patients the aorta and main branches were affected. Cerebrovascular disease was present in 14/24 patients (58%), pulmonary arterial involvement in 10/24 (42%), ischaemic heart disease in 5/24 (21%), visual disturbance in 12/24 (50%), renal arterial partial or complete occlusion in 12/24 (50%) and erythema nodosum in 5/24 (21%). Using the aortographic classification system 7/24 (30%) patients were Numano's type I, 1/24 (4%) were IIa, 1/24 (4%) were IIb, 0/24 (0%) were III, 2/24 (8%) were IV and 13/24 (54%) were V.

HLA-gene frequencies

HLA-A and -B gene frequencies (gf) in TA patients and normal controls are shown in Tables I and II. We found significantly increased frequencies of HLA-Bl5 (p=0.009, pC=0.020, OR = 3.24, EF=11.9%) and HLA-B52 (p = 0.008, pC=0.027, OR=5.16, EF=7.7%) in TA patients compared to healthy controls. On the other hand, a decreased frequency of the HLA-A24 allele in patients when compared to controls (p=0.035, pC=NS, PF=11.1%) was found. Distribution of the HLA-DR and HLA-DQB alleles was similar between patients and normal controls (data not shown).

HLA alleles and organ involvement

After comparing the results from the patients with healthy controls, we then compared the differences between those patients who had other certain

Table I. Gene frequencies (gf) of HLA-A alleles in Takayasu's arteritis patients and normal controls*.

	Patients (n=26)		Controls (n=99)					
Allele	n	gf	n	gf	p	pC	OR	PF
A1	4	0.076	15	0.075	—	—	—	—
A2	20	0.384	56	0.282	—	—	—	—
A3	4	0.076	12	0.066	—	—	—	—
A11	3	0.057	9	0 045	—	—	—	—
A19	1	0.019	0	0.000	—	—	—	—
A24	2	0.038	29	0.146	0.035	NS	0.23	11.1
A26	2	0.038	9	0.045	—	—	—	—
A29	4	0.076	9	0.045	—	—	—	—
A31	5	0.096	14	0.070	—	—	—	—
A34	1	0.019	2	0.010	—	—	—	—
A36	1	0.019	0	0.000	—	—	—	—
A68	4	0.076	24	0.121	—	—	—	—
A74	1	0.019	0	0.000	—	—	—	—

*(gf)= Gene frequency, pC= p corrected, NS= not significant, PF= Preventive Fraction (%).

Table II. Gene frequencies (gf) of HLA-B alleles in Takayasu's arteritis patients and normal controls. *

	Patients (n=26)		Controls (n=99)					
Allele	n	gf	n	gf	p	pC	OR	EF
B7	6	0.115	12	0.060	—-	—-	—-	—-
B8	1	0.019	7	0.035	—-	—-	—-	—-
B14	1	0.019	14	0.070	—-	—-	—-	—-
B15	9	0.173	12	0.060	0.009	0.020	3.24	11.9
B35	4	0.076	26	0.131	—-	—-	—-	—-
B39	4	0.079	27	0.136	—-	—-	—-	—-
B40	4	0.076	20	0.101	—-	—-	—-	—-
B41	1	0.019	2	0.010	—-	—-	—-	—-
B44	5	0.096	16	0.080	—-	—-	—-	—-
B45	1	0.019	1	0.005	—-	—-	—-	—-
B48	1	0.019	0	0.000	—-	—-	—-	—-
B50	1	0.019	2	0.010	—-	—-	—-	—-
B51	6	0.115	14	0.070	—-	—-	—-	—-
B52	5	0.096	4	0.020	0.008	0.027	5.16	7.7
B53	1	0.019	2	0.010	—-	—-	—-	—-
B58	2	0.038	1	0.005	0.048	NS	7.88	3.3

*(gf)= Gene frequency, pC= Yates corrected p value, EF= Etiologic fraction (%).

Table III. Gene frequencies (gf) of the relevant HLA-A, HLA-B and HLA-DR alleles in Takayasu's arteritis patients with and without other organ involvement.*†‡

HLA	Involvement		Non-Involved		p	pC	OR	EF
SAH	n=5	gf	n= 19	gf				
DR14	4	0.400	1	0.026	0.005	0.004	24.6	38.3
Pulmonary	n= 10	gf	n= 14	gf				
A2	11	0.550	7	0.250	0.034	0.036	3.67	40.0

* This analysis included 24 TA patients.
† (gf)= Gene frequency, pC= Yates corrected p value, EF= Etiologic fraction (%),SAH= Systemic arterial hypertension.
‡ Cerebral, ocular, cardiac, renal and erythema nodosum involvement were also studied and statistically analyzed, and none of them showed statistically significant differences.

organ involvement and those who did not (Table III). Regarding systemic arterial hypertension, we found a statistically significant difference in the frequency of HLA-DR14, in which the hypertense Takayasu's patients gene frequency was increased compared with the non-hypertense patients (p = 0.005, pC=0.004, OR=24.6, EF= 38.3%). Patients with pulmonary involvement had an increased HLA-A2 gene frequency compared with the non-pulmonary damaged patients (p=0.034, pC=0.036, OR=3.67, EF=40.0%). Pulmonary involvement refers to an angiographically demonstrated compromise of the primary branches of the pulmonary artery, without parenchymal disease. The distribution of the HLA alleles in patients with brain, ocular, cardiac or renal damage or erythema nodosum did not show statistically significant differences.

With regard to age at onset, we found that patients whose ages ranked between 11-20 years were mostly HLA-DR8 (8/18, 44%), and those between 21-30 years were mostly HLA-DR4 (8/18, 44%); however, no statistically significant difference was found (data not shown).

Discussion

There is evidence that genetic factors are involved in the development of Takayasu's arteritis and that ethnicity strongly influences such genetic predisposition. Some major histocompatibility complex (MHC) genes have been associated with genetic susceptibility to this disease in several populations. HLA-B52 is associated with the disease in India, Thailand, Korea, and Japan; HLA-B39 allele and HLA-B52, DRB1*1501, DRB5*0102, DQA1*0103, DQB1*0601, DPA1*02, DPB1*0401 haplotype have been associated in the Japanese population (19-23). Preliminary studies in 12 Mexican patients showed an association with the HLA-DR6 allele and also a high frequency of the HLA-B39 allele (28). Analysis was carried out using serological techniques; in the present study using high resolution DNA typing techniques in a large number of patients, we observed only a moderately increased frequency of this allele (gf=0.211 for DR13 and DR14 in patients vs. 0.156 in control s); this was not statistically significant. More recent data point to the direct participation of the HLA-B alleles in susceptibility to the disease. Studies in Asian populations suggest an association with the HLA-B*5201 and HLA-B*3902 alleles, which share residues at positions 63 (glutamic acid) and 67 (serina) (23). Sequencing of HLA-B alleles in Mexican patients showed a high percentage of alleles that share one or two of the mentioned residues with Asian alleles; these include subtypes of HLA-B 15 and -B52. In the present study we detected an increased frequency of HLA-B15 and HLA-B52 alleles and the most important association detected at the present time in Mexican patients is with HLA-B52 (OR=5.16). The HLA-B52 reported subtype bears glutamic acid at position 63 and serine at position 67, and 27 out of 37 reported B15 subtypes present at least glutamic acid or serine in these positions. These positions belong to one of the pockets (pocket B) which is involved in the binding of the antigen in the HLA molecule (23) and may determine the susceptibility to Takayasu's arteritis via binding and presenting a yet unknown disease-related antigen. When we compared HLA differences

between TA patients with certain particular organ involvement and patients who did not, the analysis showed an important correlation between the HLA-DR14 allele and the presence of systemic arterial hypertension and between HLA-A2 and pulmonary damage in Mexican patients. The DR14 is a DR6 subtype that in the previous study in Mexican patients was associated with the susceptibility to the disease (28). On the other hand, the HLA-A2 allele associated with pulmonary damage in TA patients is the most frequent HLA-A allele detected in the Mexican population, which also includes many variants (34). The significance of these associations is not clear, particularly because of the small number of patients analyzed and further studies with larger numbers of patients are required in order to confirm the present results.

Finally, the data suggest that HLA-B15, HLA-B52 alleles and/or residues in positions 63 and 67 of the peptide-binding site of the HLA molecule could be involved in genetic susceptibility to TA, and also suggest that others class I or class II alleles could be related with specific clinical features in this peculiar disease.

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