Association of HLA-DRB5*01 with protection against cutaneous manifestations of rheumatoid vasculitis in Brazilian patients

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ABSTRACT

Objective: To evaluate the frequency of HLA classes I and II and their association with the cutaneous manifestation of rheumatoid vasculitis (RV) in Brazilian patients. **Patients and methods:** During one year we selected 130 patients with rheumatoid arthritis (RA) classified according to the American College of Rheumatology, 1987. All patients underwent a clinical and laboratory questionnaire to exclude other causes of cutaneous vasculopathy (neoplasia, infections, illicit drug use, diabetes mellitus, and tobaccoism). Seventy-three patients with any risk factor for other causes of vasculopathy were excluded. Fifty-seven without risk factors for other causes of vasculopathy were included in the study, 17 with RV according to Scott and Bacon's criteria, 1984. Demographic data, time of RA diagnosis, disease activity (DAS28), presence of rheumatoid factor, and anti-cyclic citrullinated peptide antibodies were analyzed. The HLA alleles were typed using the DNA-amplified polymerase chain reaction with low-resolution hybridization and sequence-specific primers. **Results:** The comparison between the 40 patients without RV and the 17 patients with RV showed an increased frequency of HLA-B*14 (Pc = 0.168) and HLA-Cw*08 (Pc = 0.084) in patients with RV and an increased frequency of HLA-DRB5*01 (Pc = 0.048) in patients without RV. **Conclusion:** The HLA-DRB5*01 may confer protection against that extra-articular manifestation of RA.

Keywords: rheumatoid vasculitis, HLA antigens, disease susceptibility, protection.

INTRODUCTION

Autoimmune diseases are often associated with human leukocyte antigen (HLA) genes, which are encoded in the major histocompatibility complex. HLA molecules participate in antigen presentation and cell response playing an important role in the immune response.[1] Their effect on T cell repertoire is striking and crucial in the pathogenesis of rheumatoid arthritis (RA), especially in a more severe form of disease, interacting with the HLA system.[2] HLA-B8 combined/linked DR3,[3] HLA-DRB1*04,[4–10] HLA-Cw*03,[2] and HLA-DQB1*03[11,12] have been associated with rheumatoid vasculitis (RV) in populations with homogeneous racial/ethnics characteristics. We recently studied some cases of autoimmune vasculitis and HLA and could note that HLA-DR alleles may influence their clinical expression and outcome in a mixed population.[13]

RV is a systemic manifestation of RA that can affect many organs, but our aim was to evaluate cutaneous manifestation. We aimed to evaluate the frequency rate of HLA classes I and II as well the possibility of association with cutaneous manifestation of RV in Brazilian patients, checking their relationship with demographic factors, time of RA diagnosis, disease activity, and laboratory data in comparison with RA patients without RV or with other causes of vasculopathy, because there are no studies relating RV and HLA in mixed population.

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PATIENTS AND METHODS

Patients and controls

One hundred thirty patients in treatment of RA using disease-modifying antirheumatic drugs (DMARDs) during the year of 2006, performing routine visits in the Ambulatory Service of Rheumatology at the Hospital Universitário, Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil, were prior selected. They agreed to participate in the study after reading and signing the Consent Form approved by the Medical Ethics Committee. A clinical and laboratory questionnaire was applied for exclusion of other causes of cutaneous vasculopathy, such as neoplasia, infections, illicit drug use, diabetes mellitus, and tobaccoism.

The questionnaire was applied for all patients and 73 were excluded because of history of smoking more than 20 cigarettes/day, glucose greater than 126 mg/dL in two takes, positive serology for hepatitis B or C, illicit drug use, and/or neoplasia. Of these, four patients had episodes of deep cutaneous ulcer and digital infarcts, but this was not seen during the selection, and three patients had such clinical affections visible during the selection. According to the Scott and Bacon's criteria,[14] 17 patients were diagnosed with RV due to the presence of deep cutaneous ulcers associated with digital infarcts visible at the time of selection and without clinical data or image for visceral involvement of vasculopathy. Forty patients never showed episodes of cutaneous or visceral vasculopathy during one year of study.

The patient's age ranged from 17–78 years (46 females), comprising 32 Caucasians, self-described to be of Western and Southern European ancestries; 14 Mulattoes (Caucasian and Black admixtures); and 11 Blacks, historically mostly of African ancestries (Bantu, Benin and Senegal).[15,16] RA was diagnosed in accordance to the American College of Rheumatology (ACR) criteria, 1987.[17]

The same clinical and laboratory questionnaire also included an analysis of records containing demographic data, such as gender, age, and race/ethnicity, as well as time of clinical diagnosis of initial RA and EULAR Disease Activity Score-28 (DAS28); and laboratory data, such as rheumatoid factor (RF), and antibodies to cyclic citrullinated peptide (anti-CCP). The calculated values of DAS28 were considered as > 5.1 for very active disease, as > 3.2 ≤ 5.1 for moderate disease activity, and as ≤ 3.2 for inactive disease.[18]

All 57 patients were submitted to HLA classes I and II genotyping, being statistically analyzed by the Biostatistics Service at the Research Commission of the Faculty of Medical Sciences, Unicamp, Campinas, SP, Brazil.

Genotyping

HLA-class I genotyping was performed by the polymerase chain reaction amplification (PCR) technique using sequence of specific primers (One Lambda Inc., CA, UK). HLA-class II genotyping was performed by the PCR amplification technique using sequence of specific primers (low resolution – DYNAL, Biotech Ltd., UK).

Detection of autoantibodies

Enzyme immunoassays using anti QUANTA Lite™ CCP3 IgG (INOVA) detected antibodies semiquantitatively, and the values were considered positive when > 22 IU/dL.

Detection of rheumatoid factor

RF was detected by the quantitative method of nephelometry, and the values were considered positive when > 30 IU/mL.

Statistical analysis

The frequency of alleles in patients and controls was analyzed using the Fisher's exact test. The Mann-Whitney test or χ^2 test was used for numerical variables, such as age, DAS28, gender, race/ethnicity, RF, anti-CCP and time of RA diagnosis. The statistical software used for data analysis was SPSS 17.0. The Bonferroni correction (*P*c) was used for significant statistical values, after using the Fisher's exact test. P values were considered significant when < 0.05.

RESULTS

Most patients with RV were Caucasian female patients, but the demographic showed no statistical difference about gender, age, and race/ethnicity between the two groups of patients. Regarding time of RA diagnosis there was also no statistical difference between the groups. The average time of RA diagnosis in the 17 patients with RV was 13.1 years. Since most patients with RV were positive for RF and anti-CCP, there was no statistical difference between the groups as well. Regarding DAS28, there was no statistical difference (*P*c = 0.057) among patients with RV in comparison with patients without RV (Table 1).

The patients expressed HLA-A (*01/*02*/*03/*11/*23/*24/*25/*26/*29/*30/*32/*34/*66/*68/*74/*80), HLA-B (*07/*08/*13/*15/*18/*27/*35/*37/*38/*39/*40/*42/*44/*45/*46/*47/*49/*50/*51/*52/*53/*55*56/*57/*58/*59*81), HLA-C (*01/*02/*03/*04/*05/*06/*07/*12/*14/*15/*16/*17/*18), HLA-DRB1 (*01/*03/*04/*07/*08/*09*10/*1

1/*12/*13/*14), HLA-DRB3 (*01), HLA-DRB4 (*01), and HLA-DQB1(*02/*03/*04/*05/*06).

However, only HLAB*14 (P = 0.006), HLA-Cw*08 (P = 0.006), and HLA-DRB5*01 (P = 0.048) typings were statistically significant by the Fisher's exact test. The Bonferroni correction was applied for HLA-B*14, HLA-Cw*08, and HLA-DRB5*01, which were statistically significant by the Fisher's exact test, showing HLA-B*14 (*Pc* = 0.168) and HLA-Cw*08 (*Pc* = 0.084) in patients with RV and HLA-DRB5*01 (*Pc* = 0.048) in patients without RV (Table 2).

Table 1
Comparison of demographic, clinical and laboratory data in patients with RA/RV (n = 57)

Variables	RA (n = 40)	RV (n = 17)	P	*Pc*
Gender			ns	–
Female	31 (77.5%)	15 (88.2%)		
Male	9 (22.5%)	2 (11.8%)		
Race/ethnicity			ns	–
Caucasian	20 (50.0%)	12 (70.5%)		
Mulatto	11 (25.5%)	3 (17.6%)		
Black	9 (22.5%)	2 (11.7%)		
Age (years)			0.337	–
mean	54.7	57.8		
min-max	17–78	32–71		
Time RA (years)			0.205	–
mean	11.0	13.1		
min-max	4–19	4–9		
DAS28			0.001*	0.057**
mean	4.9	5.6		
min-max	2.8–6.1	5.3–6.7		
RF	30 (75.0%)	16 (94.1%)	ns	–
Anti-CCP	27 (67.5%)	13 (76.5%)	ns	–

RA: rheumatoid arthritis; RV: rheumatoid vasculitis; RF: rheumatoid factor; anti-CCP: cyclic citrullinated peptide; DAS28: disease activity score; min: minimum; max: maximum; ns: not significant (chi-square test). *P < 0.05 (Mann-Whitney test); **Pc (Bonferroni correction).

Table 2
Frequency (%) of alleles in patients with RA/RV (n = 57)

Allele frequency	RA (n = 40)	RV (n = 17)	P	*Pc*
B*14	0 (0.0%)	4 (23.5%)	0.006*	0.168**
Cw*08	0 (0.0%)	4 (23.5%)	0.006*	0.084**
DRB5*01	9 (22.5%)	0 (0.0%)	0.048*	0.048**

RA: rheumatoid arthritis; RV: rheumatoid vasculitis. *P < 0.05 (Fisher's exact test); **Pc < 0.05 (Bonferroni correction).
HLA-B: *07/*08/*13/*14/*15/*18/*27/*35/*37/*38/*39/*40/*42/*44/*45/*46/*47/*49/*50/*51/*52/*53/*55/*56/*57/*58/*59/*81
HLA-Cw: *01/*02/*03/*04/*05/*06/*07/*08/*12/*14/*15/*16/*17/*18
HLA-DRB5: *01

DISCUSSION

In this study, RV was observed in a higher proportion of patients (29.8%) when compared with other studies (2.1%),[19] because our sample was small before the patients which agreed to participate in the study during one year and after excluding patients with a risk factor for other causes of cutaneous vasculopathy. The female gender was prevalent in both groups, due to RA being a more common disease in women.[4] There was no statistical difference regarding gender, age, and race/ethnicity between the two groups. The female gender was more frequent in our patients with RV, differing from other studies, which showed a male predominance.[20,21]

There is no previous data on race/ethnicity in RV,[20,21] but Caucasians were prevalent in the present study in both groups, since this is the predominant race/ethnicity in the Southeastern region of Brazil,[15,16] where the study took place. Regarding time of RA diagnosis, there was also no statistical difference in the groups. The average time of RA diagnosis in 17 patients with RV was 13.1 years, which proved to be similar to other studies that show the RV emergence after more than 10 years of RA.[21,22] Regarding the DAS28, there was no statistical difference between patients with and without RV after the Bonferroni correction. RF and anti-CCP were positive for most patients with RV, in agreement with other studies;[4,23–26] however, there was no statistical difference between the two groups.

The importance of detecting HLA in patients with RA in a mixed population has been demonstrated in other studies;[24,27] however, until the publication of the present study, there were no articles correlating HLA and RV in patients with RA in the Brazilian population. Regarding the association between HLA and RV, articles have been previously published in homogeneous populations showing correlation with HLA-B8 combined/linked DR3,[3] HLA-DRB1*04,[4–10] HLA-Cw*03,[2] and HLA-DQB1*03,[11,12] while the HLA-DRB1 molecule is more evident in most studies, possibly because it is expressed at a level five times higher than its paralogues HLA-DRB3, DRB4 and DRB5.[28]

This study demonstrated an increased frequency of HLA-B*14 and HLA-Cw*08 alleles in patients with RV, which was not previously reported. However, this increased frequency showed statistical significance only by the Fisher's exact test, which was not observed by the Bonferroni correction. The increased frequency of HLA-DRB5*01 appeared to provide protection against RV in our study, which was not observed in any other studies, with statistical significance both by the Fisher's exact test and the Bonferroni correction.

A study was published correlating the HLA-DRB5 null with worsening of autoimmune disease,[28] which was not observed in our study, where the *01 allele was amplified and had associated protection. The presence of HLA-DRB5 is linked with allelic variants of HLA-DRB1, otherwise it is omitted.[29]

We concluded that, although the Brazilian population is usually mixed, the findings are not similar to those found in homogeneous populations with RV, the HLA-DRB1 being the primary susceptibility gene for protection. RV was more evident in female patients, Caucasian race, RF, and anti-CCP positive, but the data were not statistically significant. HLA-B*14 and HLA-Cw*08 were not statistically significant regarding susceptibility to RV. Considering that HLA-DRB5*01 is significantly less frequent in patients with RV, it may confer protection against this extra-articular manifestation of RA. The presence do HLA-DRB5 is due to the allelic variants of HLA-DRB1, which may behave differently in a mixed population with a higher allelic variance than in homogeneous populations. A multicenter study in the Southeast Brazilian region will enable to increase the sample of patients and thus observe the expression and protection of HLA found.

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