

HLA-DRB1 alleles in juvenile-onset systemic lupus erythematosus: renal histologic class correlations

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Abstract

Human leukocyte antigens (HLA) DRB1*03 and DRB1*02 have been associated with systemic lupus erythematosus (SLE) in Caucasians and black populations. It has been observed that certain HLA alleles show stronger associations with SLE autoantibodies and clinical subsets, although they have rarely been associated with lupus renal histologic class. In the present study, HLA-DRB1 allele correlations with clinical features, autoantibodies and renal histologic class were analyzed in a cohort of racially mixed Brazilian patients with juvenile-onset SLE. HLA-DRB1 typing was carried out by polymerase chain reaction amplification with sequence-specific primers using genomic DNA from 55 children and adolescents fulfilling at least four of the American College of Rheumatology criteria for SLE. Significance was determined by the chi-square test applied to 2 x 2 tables. The HLA-DRB1*15 allele was most frequent in patients with renal, musculoskeletal, cutaneous, hematologic, cardiac, and neuropsychiatric involvement, as well as in patients positive for anti-dsDNA, anti-Sm, anti-U1-RNP, and anti-SSA/Ro antibodies, although an association between HLA alleles and SLE clinical features and autoantibodies could not be observed. The HLA-DRB1*17, HLA-DRB1*10, HLA-DRB1*15, and HLA-DRB1*07 alleles were significantly higher in patients with renal histologic class I, class IIA, class IIB, and class V, respectively. The present results suggest that the contribution of HLA-DRB1 alleles to juvenile-onset SLE could not be related to clinical or serological subsets of the disease, but it may be related to renal histologic classes, especially class I, class II A, class II B, and class V. The latter correlations have not been observed in literature.

Key words

- Systemic lupus erythematosus
- Human leukocyte antigen
- Lupus nephritis
- Juvenile-onset lupus
- HLA-DRB1 alleles

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Publication supported by FAPESP.

Received August 13, 2006
Accepted January 22, 2007

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by multisystem organ involvement and autoantibodies production (1). Genetic factors are likely to be important both in determining the overall susceptibility to SLE and influ-

encing the remarkable clinical heterogeneity of disease presentation and autoantibody production observed in affected subjects (2). Human leukocyte antigen (HLA) DRB1*02 and the allelic variant DRB1*15 have been associated with SLE in blacks and Asians and HLA-DRB1*03 has been associated with lupus in Caucasians, but clear associations

with HLA and SLE could not be demonstrated in racially mixed populations (2-5). It is recognized that certain HLA alleles show stronger association with autoantibody repertoires and clinical subsets than with SLE itself. Some studies have observed an association with HLA alleles and cutaneous or cardiac involvement (6). In addition, most studies showed an association between HLA-DRB1*02 and DRB1*15 and lupus nephritis, although HLA alleles have rarely been associated with SLE renal histologic class (3,4,6,7). The association with HLA and SLE in children appears to be similar to that observed in adults, but clinical involvement, autoantibodies and renal histologic class frequencies are quite different in these two groups (8).

We conducted this study to determine HLA-DRB1 allele associations with SLE clinical subsets, autoantibodies and renal histologic class in a cohort of Brazilian children and adolescents.

Patients and Methods

Patients

We studied 55 consecutive Brazilian children and adolescents with SLE attending the Rheumatology Unit, Children's Institute, Faculty of Medicine, University of São Paulo. All patients met the American College of Rheumatology classification criteria for SLE (9). The study was approved by the local Ethics Committee and all patients or persons responsible gave written informed consent to participate. Patient mean age at disease onset was 10.4 years (range, 1-17 years), mean follow-up period was 3.5 years (range, 1-13 years) and 46 patients (84%) were females (female:male ratio of 5.1:1). Clinical and serologic characteristics were recorded on a specific protocol form. The definitions of the clinical features were based on the American College of Rheumatology criteria (9). Forty-three patients underwent a renal

biopsy and renal histologic class was established according to the World Health Organization (WHO) classification of lupus nephritis (1,10).

Laboratory investigations

Serologic tests were performed in the same laboratory for all patients. Antinuclear antibodies were determined by indirect immunofluorescence using rat liver sections or human cell line HEP-2 as substrate (sera diluted 1/40). Anti-dsDNA antibodies were determined by indirect immunofluorescence with *Crithidia luciliae* as substrate (sera diluted 1/10). Precipitating antibodies to extractable nuclear antigens, including SSA/Ro, SSB/La, U1-RNP and Sm were detected by enzyme-linked immunosorbent assay. The anti-ribosomal P antibody was also detected by enzyme-linked immunosorbent assay. A total of 14 HLA-DRB1 alleles (*01, *15, *16, *17, *18, *04, *11, *12, *13, *14, *07, *08, *09, *10) were identified by polymerase chain reaction amplification with sequence-specific primers. The method was based on the procedure developed by Olerup and Zetterquist (11). Genomic DNA was extracted from peripheral blood mononuclear cells by the DTAB/CTAB method (12). Briefly, the buffy coat from 5 mL blood was vigorously mixed with 12% DTAB solution (12% DTAB, 2.25 M NaCl, 150 mM Tris, pH 8.6, 75 mM EDTA) and incubated for 5 min at 68°C. After the addition of 2 volumes of chloroform, samples were vigorously mixed and centrifuged for 2 min at 10,000 g for separation into three phases. The upper phase was transferred to a fresh tube containing 2 volumes of 0.5% CTAB (0.5% CTAB, 0.04 M NaCl) and DNA was precipitated and further washed with 99.5% ethanol. PCR was carried out for 35 cycles under the following conditions: 200 ng DNA, 10 mM Tris-HCl, pH 8.4, 50 mM KCl, 2 mM MgCl₂, 0.001% gelatin, 0.2 mM dNTP, 20 pmol of each primer, 2 U Taq DNA poly-

merase, and H₂O up to 55 µL were denatured for 1 min at 94°C, annealed for 1 min at 55°C, with an extension at 72°C for 1 min. The PCR products were identified by 1.2% agarose gel electrophoresis.

Statistical analysis

To compare the HLA allele frequencies of SLE patients with and without the various clinical characteristics, autoantibodies and renal histologic class, the conventional chi-square test with Yates' correction for continuity or, when appropriate, the Fisher exact test (two-tailed) were used. The odds ratio was calculated with 2 x 2 contingency tables. The 95% confidence intervals were obtained using Cornfield's approximation. The P value was corrected for the number of alleles tested. The level of significance was set at 95% (13).

Results

The following clinical manifestations and autoantibodies were detected in this group of 55 children and adolescents with SLE: cutaneous involvement (N = 53, 96%), musculoskeletal involvement (N = 42, 76%), lung involvement (N = 13, 24%), cardiac involvement (N = 23, 42%), and neuropsychiatric involvement (N = 25, 45%); renal (N = 45, 82%) and hematologic (N = 47, 85%) abnormalities; 55 (100%) had anti-nuclear antibodies, 38 (69%) anti-dsDNA, 17 (31%) anti-Sm, 20 (36%) anti-U1-RNP, 19 (34%) anti-SSA/Ro, and 6 (11%) anti-SSB/La antibodies. Eleven patients were tested for the anti-ribosomal P antibody, which was positive in 3 (27%).

HLA-DRB1*15, an allelic variant of DRB1*02, was more frequently detected in SLE patients with renal, musculoskeletal, cutaneous, hematologic, cardiac, and neuropsychiatric features compared with SLE patients without these clinical features, although a statistical significant association between

HLA alleles and SLE clinical involvement could not be demonstrated (Table 1). As expected, the HLA-DRB1*15 allele was more frequent in patients with positive anti-dsDNA, anti-Sm, anti-U1-RNP, and anti-SSA/Ro antibodies; HLA-DRB1*04 and HLA-DRB1*07 were most frequent in patients with positive anti-SSB/La antibody; the HLA-DRB1*13 allele was more frequent in patients with positive anti-ribosomal P antibody, although the differences were not statistically significant (Table 2).

The patterns of glomerular damage seen in 43 renal biopsies according to the WHO classification of lupus nephritis were: minimal mesangial glomerulonephritis in 6 patients (14%) (class I), mesangial glomerulonephritis A in 6 (14%) (class IIA), mesangial

Table 1. Relative HLA phenotype frequency according to clinical manifestations in 55 children and adolescents with systemic lupus erythematosus.

Clinical manifestations	HLA	+	(%)	-	(%)	OR	95%CI
Nephritis	DRB1*15	31.1%	40.0%	0.68	0.14-3.46		
Musculoskeletal involvement	DRB1*15	28.5%	54.5%	0.47	0.11-2.0		
Cutaneous involvement	DRB1*15	33.9%	0.0%	-	-		
Hematologic abnormalities	DRB1*15	31.9%	37.5%	0.78	0.13-4.84		
Lung involvement	DRB1*04	38.4%	14.3%	3.75	0.75-19.3		
Cardiac involvement	DRB1*15	30.4%	34.4%	0.84	0.23-3.04		
Cardiac involvement	DRB1*07	30.4%	21.8%	1.56	0.39-6.26		
Neuropsychiatric involvement	DRB1*15	32.0%	33.3%	0.94	0.26-3.37		
Neuropsychiatric involvement	DRB1*13	32.0%	56.7%	2.35	0.56-10.2		

HLA = human leukocyte antigen; OR = odds ratio; 95%CI = 95% confidence interval. All P values were ≥0.10.

Table 2. Relative HLA phenotype frequency according to type of autoantibodies in 55 children and adolescents with systemic lupus erythematosus.

Autoantibodies	HLA	+	(%)	-	(%)	OR	95%CI
Anti-dsDNA	DRB1*15	31.5%	22.2%	0.85	0.22-3.34		
Anti-Sm	DRB1*15	47.1%	26.3%	2.49	0.65-9.77		
Anti-U1-RNP	DRB1*15	45.0%	25.7%	2.36	0.64-8.91		
Anti-SSA/Ro	DRB1*15	36.8%	30.5%	1.33	0.35-4.99		
Anti-SSB/La	DRB1*04	33.3%	18.4%	2.22	0.24-18.1		
Anti-SSB/La	DRB1*07	33.3%	24.5%	1.54	0.17-11.9		
Anti-ribosomal P antibody	DRB1*13	66.6%	21.2%	8.40	0.51-262.3		

HLA = human leukocyte antigen; OR = odds ratio; 95%CI = 95% confidence interval. All P values were ≥0.13.

glomerulonephritis B in 8 (19%) (class IIB), focal proliferative glomerulonephritis in 4 (9%) (class III), diffuse proliferative glomerulonephritis in 4 (9%) (class IV), and membranous glomerulonephritis in 15 (35%) (class V).

HLA-DRB1*17, a subtype of DRB1*03, was positively associated with renal histologic class I ($P = 0.034$) compared with SLE patients belonging to other renal histologic classes. Similarly, HLA-DRB1*10 was associated with class IIA ($P = 0.03$), HLA-DRB1*15 with class IIB ($P = 0.011$) and HLA-DRB1*07 with class V ($P = 0.012$), although the differences were not statistically significant after correction for the number of comparisons (Table 3).

Discussion

Multiple loci can contribute to SLE susceptibility and disease pathogenesis is expressed as a complex genetic interaction that is undoubtedly influenced by environmental factors (2). Genetic studies of SLE are difficult because the disease is heterogeneous and can be divided into several clinical and immunologic subsets, each with its own genetic background (2). Furthermore, genes predisposing to SLE may vary according to

patient ethnic group (2,3).

Several studies, including association and family investigations, have confirmed the linkage of the SLE and MHC region in humans. The HLA-DRB1*03 allele has been associated with SLE in Caucasian populations while the HLA-DRB1*02 allele and the HLA-DRB1*15 and HLA-DRB1*16 subtypes have been associated with SLE in black and Asian ethnic groups (2-4,6,14-21). The association of HLA-DRB1*07 and HLA-DRB1*13 with lupus was observed in a few studies, such as that by Wilson et al. (22) on African Americans and that by Cowland et al. (23) in Denmark. Barron et al. (24) showed similar associations in children with SLE: HLA-DR3 (DRB1*0301) in Caucasians and HLA-DR2 (DRB1*1503/DRB1*1501), DRB5*0101, DQA1*0102, and DQB1*0602 in blacks. In Taiwanese patients with juvenile-onset SLE there was an association with HLA-DRB1*1602 (21). Silva and Donadi (25), studying 93 Brazilian SLE patients aged 13 to 66 years, observed a higher frequency of HLA-DRB1*03, a result that differs from that observed in our previous study on 55 children and adolescents with SLE in which HLA-DRB1*15 was the most frequent allele (5), and from that reported by Fernandes et al. (26) in a study on 56 adult patients with SLE in which HLA-DRB1*02 was the most frequent allele.

It has been suggested that certain MHC haplotypes show stronger association with SLE clinical manifestations and autoantibodies than with the disease itself (3,4). In infants, SLE has been reported to be clinically and serologically different, with a higher frequency of renal involvement and mortality compared to adults (1,8). These differences raise some questions regarding the possibility that childhood SLE could be a disease with its own genetic background.

We observed that the HLA-DRB1*15 allele was most frequent in patients with cutaneous, cardiac, neuropsychiatric, renal,

Table 3. Relative HLA phenotype frequency according to renal histologic class in 55 children and adolescents with systemic lupus erythematosus.

Renal histologic class	HLA	+	(%)	-	(%)	Pc value	OR	95%CI
Class I	DRB1*17	50.0%	10.2%	0.47	8.80	1.03-82.4		
Class IIA	DRB1*01	33.3%	16.3%	-	2.56	0.27-21.46		
	DRB1*11	33.3%	10.2%	-	4.40	0.42-42.56		
	DRB1*07	33.3%	24.5%	-	1.54	0.17-11.94		
	DRB1*10	33.3%	2.1%	0.42	24.0	1.25-878.2		
Class IIB	DRB1*15	75.0%	25.5%	0.15	8.75	1.30-73.7		
Class III	DRB1*15	50.0%	31.5%	-	2.19	0.20-24.6		
	DRB1*07	50.0%	23.5%	-	3.25	0.28-37.5		
Class IV	DRB1*17	50.0%	11.7%	-	7.50	0.60-98.9		
	DRB1*08	50.0%	13.7%	-	6.29	0.51-79.6		
Class V	DRB1*07	53.0%	15.0%	0.16	6.48	1.43-31.0		

HLA = human leukocyte antigen; OR = odds ratio; 95%CI = 95% confidence interval; Pc value = P value corrected for 14 comparisons.

and hematologic involvement, although an association could not be demonstrated. Doherty et al. (6) described an association with HLA-DR15 and SLE skin involvement in Chinese patients and Reivelle et al. (27) demonstrated an association with HLA-DR53 and HLA-DQA1 and malar erythema photosensitivity. Doherty et al. (6) also reported an association with the HLA-DR7 and HLA-DR9 alleles and cardiac involvement in Chinese patients. None of our patients with cardiac involvement presented the HLA-DRB1*09 allele. In the Brazilian study of Silva and Donadi (25), HLA-DR3, HLA-DR9 and HLA-DQ2 were the most frequent alleles in SLE patients with neuropsychiatric involvement, a result that differs from that observed in the present study. Fronck et al. (7) and Doherty et al. (6) described an association with HLA-DR2 and SLE nephritis in Americans. Lupus nephritis has also been associated with the HLA-DR3, HLA-DR15 and HLA-DR16 alleles in different ethnic groups (6,7,28). There is no description in the literature of an association between HLA alleles and SLE hematologic involvement. Galeazzi et al. (28) described an association between HLA-DRB1*03 and lung involvement in SLE. Alternatively, HLA-DRB1*04 was the most frequent allele in our patients with lung abnormality.

Some associations have been described concerning the HLA-DR alleles and SLE autoantibodies. The anti-dsDNA antibody has been associated with the HLA-DR2, DR3, DR7, and DR15 alleles (2,28). The anti-Sm antibody has been associated with the HLA-DR2, HLA-DR4 and HLA-DR7 alleles, the anti-U1-RNP antibody with HLA-DR4, and the anti-SSA/Ro and anti-SSB/La antibodies with the HLA-DR2 and HLA-DR3 alleles (3,4,28-32). The HLA-DRB1*15 allele was most frequent in our patients with positive anti-dsDNA, anti-Sm, anti-U1-RNP, and anti-SSA/Ro antibodies, although an association could not be observed. Additionally, the anti-ribosomal P antibody has been found

to be associated with the HLA-DR4 and DR2 (DRB1*1501 or DRB1*1503) alleles (2,33). None of our patients positive for anti-ribosomal P presented the HLA-DR4 or HLA-DR2 allele. For the interpretation of the present study it should be considered that clear associations between HLA region genes and SLE clinical and serological features could not be demonstrated because genes within or in linkage with the HLA region, such as DQ, DP, TNF- α , and C4 were not evaluated (14,16,19,28,34).

Renal histologic class is an important prognostic factor for SLE patients. Nevertheless, HLA alleles have rarely been associated with SLE renal histologic class. The renal histologic class frequencies observed in the present study were similar to those reported in the literature (1,8). Interestingly, 3 of 6 patients belonging to WHO renal histologic class I showed HLA-DRB1*17, an allelic variant of DRB1*03. HLA-DRB1*03 has been associated with SLE patients with disease onset after 35 years of age, who have less frequent and less severe renal involvement (2), possibly suggesting a protective effect of the HLA-DRB1*03 allele for lupus nephritis. On the other hand, Shankarkumar et al. (35), studying 53 patients with severe SLE who had one or more organ affected, such as kidney, brain, heart, and lungs, observed that the HLA-DRB1*03 allele was significantly increased in this group. Only 3 of the 55 children and adolescents with SLE presented HLA-DRB1*10 and 2 of them belonged to renal histologic class IIA. Eight patients belonged to class IIB and 6 of them presented the HLA-DRB1*15 allele, although our study could not demonstrate an association between HLA-DRB1*15 and lupus nephritis *per se*. Fifteen patients had membranous glomerulonephritis (class V) and 8 presented HLA-DRB1*07. These findings are different from those observed by Marchini et al. (36), who showed an association between HLA-DRB1*15 and severe form of lupus glomerulonephritis class III

and class IV. Interestingly, Endreffy et al. (37) observed that the DRB1*1501 allele was less frequent in patients with lupus nephritis. In contrast, HLA-DRB1*03 and DRB1*07 alleles were more frequent in patients with lupus nephritis. In addition, HLA-DR expression was significantly increased in kidney arteries and arterioles of SLE patients compared to controls, although it did not correlate with WHO renal histologic class (38). The extended HLA-A1,B8,DR3 haplotype has been associated with idiopathic membranoproliferative glomerulonephritis and with SLE (39). The individual

HLA-DR3, HLA-DR15 and HLA-DR7 components have been associated with membranous idiopathic nephropathy, membranoproliferative glomerulonephritis and idiopathic nephrotic syndrome, respectively (39). Recently, a Brazilian study observed an association between DD genotype of the angiotensin-converting enzyme and SLE renal histologic classes III and IV (40).

The present results suggest that the contribution of MHC genes to SLE cannot be only related to the clinical and serological subsets of the disease but also to its renal histologic class.

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