Antinuclear Antibodies and HLA Class II Alleles in Jamaican Patients with Systemic Lupus Erythematosus

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ABSTRACT

Objective: *The relationship between human leukocyte antigens class II (HLA) and antinuclear antibodies was investigated in Jamaican patients with Systemic Lupus Erythematosus (SLE).*

Methods: *Samples of blood of 82 patients with SLE and 75 healthy controls were tested for antinuclear antibodies using the fluorescent antinuclear antibody (FANA) test, counterimmunoelectrophoresis (CIEP) and the* Crithidia luciliae *immunofluorescence test (CL-IFT). A DNA-based HLA typing method was used to determine the frequencies of alleles of HLA-DRB1, DRB3, DRB4 and DRB5 in patients and healthy controls.*

Results: *The FANA test was positive in all of the sera from patients with SLE. Anti-dsDNA antibodies were present in 49% (40/82), anti-Sm/RNP 44% (36/82) and anti-Ro/La 43% (35/82) of the sera from SLE patients. The frequency of HLA-DR4 was significantly lower in SLE patients than in healthy controls (2/82, 2%* vs *15/75, 20%; RR = 0.12;* p *= 0.0004; CP = 0.005) but no other HLA-DRB1 SLE associations were found. A positive HLA-DR3 anti-Ro/La antibody association was found in the patients with SLE (9/21, 43%* vs *5/55, 9%; odds ratio (OR) = 7.5; CP = 0.01). In contrast, possession of HLA-DR6 was negatively associated with the absence of anti-dsDNA antibodies (9/32, 28%* vs *27/44, 61%; OR = 0.2; CP = 0.05).*

Conclusion: *The HLA-DR6 allele is associated with the absence of antinuclear antibodies and HLA-DR3 with the presence of anti-Ro/La antibodies in Jamaican patients with SLE. However, these results and those of previous studies of Jamaican patients suggest that the HLA-DR3 association with the development of SLE reported in other populations might in fact reflect the association of HLA-DR3 with anti-Ro/La antibodies. Further investigations are needed to determine whether HLA-DRB antinuclear antibody associations define clinical subsets of SLE in Jamaican patients.*

Anticuerpos Antinucleares y Aleles HLA de Clase II en Pacientes Jamaicanos con Lupus Eritematoso Sistémico

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RESUMEN

Objetivo: *Se investigó la relación entre los antígenos de leucocito humano (human leukocyte antigens o HLAs). Clase II y los anticuerpos antinucleares en pacientes jamaicanos con lupus eritematoso sistémico (LES).*

Métodos: *Se examinaron muestras de sangre de 82 pacientes con LES y 75 controles saludables para determinar la presencia de anticuerpos antinucleares, usando la prueba del anticuerpo antinuclear fluorescente (FANA), la contrainmunoelectroforesis (CIEP) y el test de inmunofluorescencia con Crithidia luciliae (CL-IFT). Un método de tipificación HLA basado en el ADN fue usado para determinar las frecuencias de aleles de HLA-DRB1, DRB3, DRB4 y DRB5 tanto en los pacientes como en los controles saludables.*

Resultados. *La prueba FANA fue positiva en todos los sueros de pacientes con LES. Anticuerpos anti-dsADN se hallaban presentes en 49% (40/82), anti-Sm/RNP en 44% (36/82) y anti-Ro/La en 43% (35/82) de los sueros de los pacientes de LES. La frecuencia de HLA-DR4 fue significativamente más baja en los pacientes con LES que en los controles saludables (2/82, 2%* vs *15/75, 20%; RR = 0.12;*

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p = *0.0004; CP = 0.005) pero no se hallaron otras asociaciones de LES con HLA-DRB1. Se halló una asociación positiva de anticuerpos HLA-DR3 anti-Ro/La en los pacientes con LES (9/21, 43% vs 5/55, 9%; odds ratio (OR) = 7.5; CP = 0.01). En contraste con ello, la posesión de HLA-DR6m estuvo asociada negativamente con la ausencia de anticuerpos anti-dsADN (9/32, 28%* vs *27/44, 61%; OR = 0.2; CP = 0.05).*

Conclusión: *El alele HLA-DR6 está asociado con la ausencia de anticuerpos antinucleares y el de HLA-DR3 con la presencia de anticuerpos anti-Ro/La en pacientes jamaicanos con LES. Sin embargo, estos resultados al igual que los de los previos estudios de pacientes jamaicanos, sugieren que la asociación HLA-DR3 con el desarrollo de LES reportado en otras poblaciones podría de hecho reflejar la asociación de HLA-DR3 con anticuerpos anti-Ro/La. Se requieren investigaciones ulteriores a fin de determinar si las asociaciones de anticuerpo antinuclear HLA-DRB definen subconjuntos de LES en pacientes jamaicanos*

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INTRODUCTION

Systemic lupus erythematosus (SLE) has been described as the paradigm of a systemic autoimmune disease, variable in expression, affecting any organ or system with a range of severity (1). It is characterized by the presence of circulating antinuclear antibodies (ANA). Its aetiology is not known but is thought to be multifactorial including genetic and environmental factors (2). The genetic loci most strongly implicated in susceptibility to SLE involve several immune response genes including the major histocompatibility complex (MHC), MHC linked and non-MHC linked genes.

The strongest SLE MHC associations are those with human leucocyte antigen (HLA) class II genes. The SLE HLA associations are heterogeneous among different ethnic groups and are better established in homogeneous populations. For example, SLE is associated with HLA-DR3 in Caucasian and Asian populations (3, 4). In patients with SLE, the HLA antinuclear antibody associations reported have been consistent across ethnic groups. These include the association between HLA-DR3, anti-Ro and anti-La antibodies, HLA-DR2 anti-Sm antibodies and HLA-DR2 and anti-dsDNA antibodies (5–8).

Previous studies have failed to demonstrate any HLA class II SLE associations in Jamaican patients (9).

PATIENTS AND METHODS

The study sample comprised 82 consecutive unrelated patients (79 females, 3 males; median age 38 years, age range 17–72 years) who fulfilled the American College of Rheumatology (ACR) criteria for the diagnosis of SLE (10). Blood donors (n = 75) were included as healthy controls in the investigations of the HLA. The study was carried out after ethical approval was obtained and patients were included after informed consent. Samples of EDTA blood (5 ml) were drawn by venepuncture from each patient and control for DNA based HLA typing. In addition, a 5 ml sample of clotted blood was collected from each patient and the sera separated and stored at -20°C until tested.

The sera were screened for ANA using the fluorescent antinuclear antibody (FANA) test as previously described (11). The antibodies to ds-DNA and extractable nuclear antigens (ENA) were detected by the *Crithidia luciliae* immunofluorescence test (CL-IFT) and counterimmunoelectrophoresis (CIEP), respectively, as previously described (12).

The DNA-based HLA typing was performed using the polymerase chain reaction sequence-specific primer (PCR-SSP) method described previously by Olerup and Zetterquist and recommended by the Twelfth Workshop for Histocompatibility testing (13, 14). Briefly, DNA was extracted from peripheral blood leukocytes treated with proteinase-K by the rapid mini-scale salting-out method, and the second exons of the DRB1, DRB3, DRB4 and DRB5 genes were amplified using 20 primer mixes previously described (13). The primers corresponded to the serologically defined series, including 17 primer mixes for DR1–DR18 and one mix each for DR52, DR53 and DR51. In each PCR reaction, a primer pair (RC 5', RC 3') was included which amplified the third intron of DRB1 genes. These two primers matched non-allelic sequences and functioned as an internal positive amplification control.

Statistical Analysis

The HLA-DRB allele frequencies in patients and control subjects were compared using the chi–square and Fisher's exact tests as appropriate. Corrected *p* values (CP*)* were calculated by multiplying *p* values by the number of alleles tested at each locus. Relative risks (RR) were calculated using Woolf's method (15, 16).

RESULTS

As shown in Table 1, all of the patients with SLE tested positive in the FANA test. The prevalence of the different antinuclear antibody specificities is also shown.

Table 1: The prevalence of antinuclear antibodies in 82 Jamaican patients with systemic lupus erythematosus

Autoantibody	Number Positive (%)
Antinuclear antibody	82/82 (100)
Anti-dsDNA	40/82 (49)
Anti-Ro/La	35/82 (43)
Anti-Sm/RNP	36/82 (44)

The frequencies of alleles of HLA-DRB1, -DRB3, -DRB4 and -DRB5 in the SLE patients and control subjects are compared in Table 2. The most frequent HLA-DRB alleles in the patients with SLE were HLA-DR6 (36/82, 44%) while HLA-DR2 (41/75, 55%) was the most prevalent in the control subjects. After correction for the number of loci tested, no significant positive HLA-DRB SLE associations were identified. However, the frequency of HLA-DR4 was significantly lower in patients with SLE than in controls (2/82, 2% *vs* 15/75, 20%; $\chi^2 = 12.51$; RR = 0.12; CP = 0.005). A significant positive association was found with HLA-DR3 and the presence of anti-Ro/La antibodies in SLE patients (9/21, 43% *vs* 5/55, 9%; $\chi^2 = 11.5$; CP = 0.01). The absence of anti-ds DNA antibodies was associated with HLA–DR6 (27/44, 61% *vs* 9/32, 28%; $\chi^2 = 8.65$; CP = 0.05). No other significant HLA-DR antinuclear antibody associations were observed.

Table 2: Comparative frequencies of HLA-DRB alleles in patients with systemic lupus erythematosus and healthy subjects

HLA	% Frequency		RR
	Patients (n = 82)	Controls (n = 75)	
DR 1	7	4	1.81
DR 2	39	55	0.52
DR 3	18	21	0.83
DR 4*	2*	20*	0.12
DR 5	13	20	0.60
DR 6	44	31	1.75
DR 7	6	4	1.53
DR 8	4	0	∞
DR 9	20	19	1.07
DR 10	5	5	1.00
DR 51	10	12	0.82
DR 52	23	11	2.42
DR 53	22	15	1.60
Blank	4	0	∞

*The frequency of HLA-DR4 was significantly lower in patients with SLE than in controls (2/82, 2% vs 15/45, 20% x2 = 12.51; RR = 0.12, CP = 0.005. CP = P value corrected by multiplying the number of alleles tested at each locus (10 alleles for DRB1 and 1 each for DRB3, DRB4, and DRB5).

DISCUSSION

The prevalence of the various antinuclear antibodies observed was comparable to that reported previously in Jamaican patients with SLE (17) and some of the subjects might also have been included in these studies. Also in keeping with previous studies of Jamaican patients with SLE, the HLA-DR associations observed in Caucasian and Black populations elsewhere were not observed in this cohort (3, 18, 19).

The negative association with HLA-DR4 and SLE found in this cohort has not been reported in other populations. The association of HLA-DR3 and anti-Ro/La antibodies observed in this cohort of Jamaican patients has also been reported in SLE patients in other ethnic groups including Caucasians, African-Americans, Mexicans and South African Blacks (3, 20–22). The HLA-DR3 anti-Ro/La association has also been reported in apparently healthy individuals with anti-Ro/La antibodies (23). The finding that HLA-DR3 is associated with the presence of anti-Ro/La antibodies in Jamaican patients with SLE is important because the HLA-DR3 association with SLE reported in several ethnic groups has not been observed previously in Jamaican patients (9).

Another significant finding is the negative association of HLA-DR6 with the presence of anti-dsDNA antibodies in the patients with SLE. Possession of the HLA-DR6 allele may describe a subset of SLE patients possibly with a milder disease course as anti-dsDNA antibodies have been associated with a more severe disease course (24). Previous studies have shown that the presence of anti-dsDNA antibodies does not correlate with any clinical characteristic of SLE in Jamaican patients (17).

In the present study, no HLA-DR associations were found with the anti-Sm/RNP antibodies. Associations between HLA-DR4 -DR3 and anti-Sm/RNP antibodies have been reported in other Black populations (6, 7).

In conclusion, HLA-DR3 is not associated with the development of SLE but with the presence of anti-Ro/La antibodies in Jamaican patients. The positive HLA-DRB antinuclear antibody associations in Jamaican patients with SLE observed in this and previous studies suggest that the HLA-DR3 SLE associations reported in other populations may in fact reflect the association of HLA-DR3 with anti-Ro/La antibodies. Further investigations are needed to determine whether HLA-DRB antinuclear antibody associations define clinical subsets of SLE in Jamaican patients.

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