HLA Class II DNA Typing in a Large Series of European Patients with Systemic Lupus Erythematosus

Correlations with Clinical and Autoantibody Subsets

MAURO GALEAZZI, GIAN DOMENICO SEBASTIANI, GABRIELLA MOROZZI, CARLO CARCASSI,
GIOVANNI BATTISTA FERRARA, RAFFAELLA SCORZA, RICARD CERVERA, ENRIQUE DE RAMON GARRIDO,
ANTONIO FERNANDEZ-NEBRO, FREDERIC HOUSSIAU, ANNA JEDRYKA-GORAL, GIUSEPPE PASSIU,
CHRYSSA PAPASTERIADES, JEAN-CHARLES PIETTE, JOSEF SMOLEN, GIOVANNI PORCIELLO, ROBERTO MARCOLONGO,
AND THE EUROPEAN CONCERTED ACTION ON THE IMMUNOGENETICS OF SLE*

Introduction

The etiopathogenesis of systemic lupus erythematosus (SLE) is complex and still largely unknown. Genetic, environmental, and hormonal factors contribute to disease susceptibility (1). The importance of genetic factors is documented by 1) the pronounced difference in concordance rates between monozygotic and dizygotic twins (15); 2) the higher disease prevalence in relatives of patients with SLE (14); 3) the higher prevalence of SLE in certain ethnic groups, like African Americans and some Native Americans (18); and 4) studies of murine SLE showing that some strains invariably develop the disease (23, 24).

In humans, several genes contribute to lupus susceptibility. Their identification has been complicated by the fact that SLE is a heterogeneous disease, both clinically and immunologically (3); in addition, many of the susceptibility genes differ across the various populations (27), and it is well recognized that genetic

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predisposition is linked to autoantibody repertoires and to clinical subsets of disease (9, 20, 31).

Many studies have focused on human leukocyte antigen (HLA) genes, mainly because they are involved in immune recognition and response, even if genetic susceptibility to SLE is in part located outside the short arm of the sixth chromosome (13, 22, 30, 33, 35, 36). Studies performed by genomic scan of multiplex lupus families confirm the pathogenetic role of the HLA system in this disease (19).

We decided to approach the problem of HLA-SLE association in a large sample of patients, all of Caucasian origin, to enable study of homogeneous subsets defined clinically and serologically. In this paper, we present the results of an immunogenetic study conducted in a large series of European SLE patients. Our objective was to determine the HLA class II allele association with SLE and its main clinical and autoantibody subsets.

Patients and Methods

Five hundred seventy-seven patients with SLE (91.2% female, 8.8% male; female:male ratio = 10:1), fulfilling 4 or more of the American Rheumatism Association (ARA) 1982 revised criteria for the classification of the disease (32), coming from 11 university hospitals of 7 European countries, were enrolled consecutively. Mean age of these patients was 37.9 ± 13.1 years (range, 13–79 yr). Mean age at the onset of the disease was 29.5 ± 12.5 years (range, 4–77 yr). Mean disease duration was 8.2 ± 3.1 years (range, 1–24 yr). To avoid regional biases, each center was asked to contribute 60 patients, with the only exception the 2 centers in Malaga, in southern Spain, that were asked to give 30 patients each. Results were available for 577 patients, whose regional distribution is depicted in Table 1.

Each of the centers recorded the clinical characteristics of the patients, including the past medical history and a complete physical examination, using specific protocol forms, and collected blood and serum samples for the immunogenetic and serologic studies. All participating centers have substantial experience in

TABLE 1. Regional distribution of 577 European patients with systemic lupus erythematosus

Country/Region	Center*	No. of Patients
Belgium	7	60
France	6	60
Greece	8	58
Southern Spain	10	27
Southern Spain	11	37
Catalonia, Spain	5	60
Poland	12	60
Northern Italy	4	60
Central/southern Italy	2	60
Sardinia, Italy	3	60
Austria	9	35

*For the center reference number see list of the centers participating in this study (European Concerted Action on the Immunogenetics of SLE) in footnote on first page of article.

the management of patients with SLE. Nevertheless, in order to minimize the possible interobserver bias, the definition of the clinical features included in the protocol forms was discussed by all participating physicians on several occasions. (For a precise definition of the clinical features, see references 5 and 29.)

Blood and serum samples were taken from patients at the time clinical information was collected and were stored at $-80\,°C$. Subsequently, they were transported in dry ice to the respective laboratories by the researchers of the clinical centers.

HLA class II typing was done at the following 3 laboratories, with the same methodology: 1) Department of Medical Genetics, Institute of Clinica Medica, University of Cagliari, Italy; 2) Immunogenetics Laboratory, National Institute for Cancer Research, Genova, Italy; 3) Laboratory of Cellular Immunology and Immunogenetics, Institute of Internal Medicine, Infectious Diseases and Immunopathology, University of Milano, Italy. To guarantee quality control of the DNA typing tests and interlaboratory procedural uniformity, there was exchange of personnel as well as of biologic material (blood samples, genetic probes) between the 3 laboratories where the tests were performed. In each patient, the following HLA loci were studied: DRB1, DRB3, DRB4, DRB5, DQA1, DQB1.

The frequency of HLA alleles in patients with SLE was compared with the frequency in a healthy Caucasian population, matched for ethnic origin, taken from the 12th International Histocompatibility Workshop (IHW) (11). Results of HLA class II allele typing of a healthy Polish population were from Jungerman et al (16).

Various serologic tests were performed, including the detection of autoantibodies that are more commonly found with SLE. Each serologic test was performed in the same laboratory for all patients (Institute of Rheumatology, University of Siena, Italy). Centralized data handling, computing, and statistical analysis took place at the Rheumatology Division, San Camillo Hospital, Rome, Italy. The data were transferred into a computerized database program (DBASE III PLUS) and were analyzed using the Statistical Package for Social Sciences (SPSS) program.

The study, approved by the Human Experimental Committee of each participating hospital, was carried out according to the principles of the Declaration of Helsinki. Informed consent was obtained from all patients.

Autoantibody detection

Antinuclear antibodies (ANA) were determined by indirect immunofluorescence using Hep-2 cells as substrate. Anti-dsDNA antibodies were determined by indirect immunofluorescence with *Crithidia luciliae* as substrate. Precipitating antibodies to extractable nuclear antigens, including Ro (SS-A), La (SS-B), U1-RNP, Sm, Jo1, SCL70, PCNA, were detected by double immunodiffusion and counterimmunoelectrophoresis using calf and rabbit thymus and human spleen extracts, as previously described (10, 20).

Other autoantibodies, such as anticardiolipin antibodies, anti-β2glycoprotein I antibodies, antineutrophil cytoplasmic antibodies, and antiganglioside antibodies, were also determined. Results of their correlation with clinical manifestation and HLA class II alleles have been published separately (7–10).

HLA typing

Molecular typing of HLA-DRB1, DRB3, DRB4, DRB5, DQA1 and DQB1 was performed according to the 11th IHW reference protocol (17) with minor variations.

DNA was extracted from peripheral blood mononuclear cells using a standard phenol/chloroform technique. HLA-DRB, DQA1, DQB1 alleles were typed by the polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSOP) method, carried out using digoxigenin (DIG)-labeled probes. The primers and the oligonucleotide probes used were those validated by the 11th IHW. Briefly, the second exon of each gene is specifically PCR amplified; the amplified fragment is immobilized on a nylon positively-charged membrane (Zeta-Probe blotting membrane, Bio-Rad, Hercules, CA) and hybridized with sequence-specific probes previously 3′-end labeled with DIG-ddUTP using terminal-transferase (Boehringer Mannheim Gmbh, Germany). An anti-DIG-alkaline phosphatase conjugate (Fab fragments) (Boehringer Mannheim Gmbh) is bound to the DIG-labeled hybrids; incubation with CSPD (Tropix, Bedford, MA) substrate allows the chemiluminescent detection of the hybrids, by exposing the membranes to a X-Omat AR autoradiography film (Eastman Kodak, Rochester, NY).

Statistical analysis

Frequencies of HLA alleles were compared among the various groups using conventional chi-square analysis with the Yates correction or, when appropriate, the Fisher exact test (2-tailed). The p value for HLA frequency differences was corrected (pc) for the number of comparisons. A p value equal to or less than 0.05 was taken as significant. An odds ratio (OR) was calculated, when the p value was significant, by using 2×2 contingency tables. When the value of a cell in the 2×2 table was 0, relative risk (RR) was calculated by Haldane's modification of Woolf's method (12):

$$RR = \frac{(a + \frac{1}{2})}{(b + \frac{1}{2})}\frac{(d + \frac{1}{2})}{(c + \frac{1}{2})}$$

This statistical analysis was performed by means of the SPSS program using the information stored in the database program.

Results

Frequency of HLA class II alleles in patients and in healthy controls

As expected, frequency of HLA-DRB1*03 was significantly higher in SLE patients compared with healthy controls (35.2 vs 19.4, $p < 10^{-6}$, pc = 1.3×10^{-5}, OR = 2.2, 95% CI [confidence intervals] = 1.8–2.9) (Table 2). In addition, DRB1*15 and DRB1*16 (DR2) were significantly associated with SLE (respectively, 27.7 vs 19.4, p = 0.003, pc = 0.04, OR = 1.6, 95% CI = 1.2–2, and 14.4 vs 10.5, p = 0.03, pc = not significant [NS], OR = 1.4, 95% CI = 1.1–2). On the contrary, DRB1*01, DRB1*04, and DRB1*11 (DR5) showed negative association with SLE. Alleles at the DRB3 locus were not associated with the disease.

Among the alleles at the DQA1 locus, only DQA1*0102 showed a positive association with SLE (42.6 vs 32.8, $p = 2 \times 10^{-4}$, pc = 0.006, OR = 1.5, 95% CI = 1.2–1.9), whereas DQA1*0101 and DQA1*03 were negatively associated (Table 3).

Some alleles at the DQB1 locus were found with significantly increased frequency in patients with SLE compared with healthy controls. They were DQB1*0502 (17.8 vs 11.6, p = 0.001, pc = 0.03, OR = 1.6, 95% CI = 1.2–2.2), DQB1*0602 (20.7 vs 15.1, p = 0.008, pc = NS, OR = 1.5, 95% CI = 1.1–1.9), DQB1*0201 (52.7 vs 39.7, $p = 2 \times 10^{-6}$, pc = 5.6×10^{-5}, OR = 1.7, 95% CI = 1.3–2.1), DQB1*0303 (5.8 vs 3.4, p = 0.05, pc = NS, OR = 1.7, 95% CI = 1–2.9), DQB1*0304 (1.9 vs 0.4, p = 0.02, pc = NS, OR = 4.3,

TABLE 2. Phenotypic frequency of HLA-DRB1 and DRB3 alleles in patients with SLE and in healthy controls

	Patients with SLE	Controls	p Value	pc (p × 13)	**OR	95% CI
DRB1	(n = 534)	(n = 958)				
*01	14.4	22.3	.003	.04	.6	.4–.8
*15	27.7	19.4	.003	.04	1.6	1.2–2
*16	14.4	10.5	.03	NS	1.4	1.1–2
*03	35.2	19.4	$< 10^{-6}$	1.3×10^{-5}	2.2	1.8–2.9
*04	14.6	21.3	.002	.03	.6	.5–.8
*11	20.2	27.3	.003	.04	.7	.5–.9
*12	3.4	1.9	NS			
*13	20.4	18.3	NS			
*14	5.4	6.3	NS			
*07	22.8	23.3	NS			
*08	5.6	5.3	NS			
*09	1.7	2	NS			
*10	2.2	2.3	NS			
DRB3	(n = 376)	(n = 670)				
*01	44.2	38.2	NS			
*02	57.5	61.1	NS			
*03	11.2	15.3	NS			

Abbreviations: SLE = systemic lupus erythematosus; pc = probability corrected for multiple comparison; OR = odds ratio; CI = confidence interval; NS = not significant.

TABLE 3. Phenotypic frequency of HLA-DQA1 and DQB1 alleles in patients with SLE and in healthy controls

	Patients with SLE	Controls	p Value	pc (p × 28)	OR	95% CI
DQA1	(n = 535)	(n = 900)				
*0101	17.2	29	$<10^{-6}$	2.8×10^{-5}	.5	.4–.7
*0102	42.6	32.8	2×10^{-4}	.006	1.5	1.2–1.9
*0103	12	14.2	NS			
*0104	4.9	.8	NS			
*0201	22.8	22.8	NS			
*03	16.6	23.7	.002	NS	.6	.5–.8
*0401	5.6	4.6	NS			
*0501	53.5	50.6	NS			
*06	3.6	.6	NS			
DQB1	(n = 535)	(n = 900)				
*0501	16.4	25	2×10^{-4}	.006	.6	.4–.8
*0502	17.8	11.6	.001	.03	1.6	1.2–2.2
*0503	5	3.7	NS			
*0504	.4	.1	NS			
*0601	3.6	4.2	NS			
*0602	20.7	15.1	.008	NS	1.5	1.1–1.9
*0603	9.3	10.1	NS			
*0604	5.8	5.3	NS			
*0605	.4	.8	NS			
*0609	.9	.1	NS			
*0201	52.7	39.7	2×10^{-6}	5.6×10^{-5}	1.7	1.3–2.1
*0301	28.2	38.6	9×10^{-5}	.002	.6	.5–.8
*0302	8.8	14.2	.003	NS	.6	.4–.8
*0303	5.8	3.4	.047	NS	1.7	1–2.9
*0304	1.9	0.4	.02	NS	4.3	1.2–16.2
*0305	.2	.1	NS			
*0401	.9	1.2	NS			
*0402	4.3	4	NS			
*08	0.9	0.5	NS			

Abbreviations: See previous tables.

95% CI = 1.2–16.2). Conversely, DQB1*0501, DQB1*0301, and DQB1*0302 showed a negative association with SLE (see Table 3).

Frequency of autoantibodies in lupus patients and correlation with HLA class II alleles

Frequencies of those autoantibodies of interest for this paper are depicted in Table 4. They are in keeping with the findings in previous studies. With the exception of anti-dsDNA (pc = 0.001), the frequencies of all other autoantibodies were not statistically different among the various centers. On the other hand, the frequency of anti-dsDNA in every patient was quite variable with time, depending mainly on the disease activity and therapy.

As already known, anti-Ro was overrepresented in haplotypes containing DR3 (36.6 in patients DRB1*03 positive vs 19 in their negative counterparts, p = 2×10^{-5}, pc = 3×10^{-4}, OR = 2.4, 95% CI = 1.6–3.7), as well as anti-La (13.1 vs 2.7, p = 1×10^{-5}, pc = 1×10^{-4}, OR = 5.5, 95% CI = 2.5–12.1)

(Table 5). Both these autoantibodies were also strongly associated with DQB1*0201 (DQw2), which is in strong linkage disequilibrium with DRB1*03, and, obviously, showed a strong association with haplotypes containing DRB1*03 and DQB1*0201. Anti-Ro, but not anti-La, was also associated with DQB1*0502 (DQw1), and with the haplotype DR2/

TABLE 4. Relative frequency of autoantibodies in patients with SLE

Specificity	Frequency
dsDNA	56.2
Ro	25.2
La	6.3
Sm	7.2
U1-RNP	18.5
Jo1	0.2
SCL70	0.2
PCNA	0.9

Abbreviations: ds-DNA = double-strand DNA; Sm = Smith; U1-RNP = U1-ribo-nuclear-proteins; SCL70 = sclero 70; PCNA = proliferating cells nuclear antigens.

TABLE 5. Relative frequency of significantly associated autoantibodies in patients positive and negative for the various HLA alleles/haplotypes

HLA	Anti-dsDNA	Anti-Ro	Anti-La	p Value	pc	OR	95% CI
DRB1*03		36.6 vs 19		2×10^{-5}	3×10^{-4}	2.4	1.6–3.7
DRB1*03			13.1 vs 2.7	1×10^{-5}	1×10^{-4}	5.5	2.5–12.1
DRB1*15	66.2 vs 52.2			0.008	NS	1.8	1.2–2.7
DQA1*0102	63.6 vs 50.4			0.004	NS	1.7	1.2–2.5
DQA1*0501			8.7 vs 3.7	0.03	NS	2.5	1.1–5.4
DQA1*0601		55.6 vs 24.2		0.006	NS	3.9	1.5–10.2
DQB1*0201		33.5 vs 18.1		8×10^{-5}	0.002	2.3	1.5–3.4
DQB1*0201			10.7 vs 2.5	3×10^{-4}	0.008	4.6	2–10.9
DQB1*0502		37.4 vs 22.7		0.005	NS	2	1.3–3-3
DQB1*0602	66 vs 53.5			0.03	NS	1.7	1.1–2.7
DRB1*03/DQB1*0201		36.7 vs 19.2		2×10^{-5}	3×10^{-4}	2.4	1.6–3.7
DRB1*03/DQB1*0201			13.3 vs 2.7	1×10^{-5}	1×10^{-4}	5.6	2.6–12.4
DR2/DQB1*0502		37.6 vs 22.8		0.006	NS	2	1.2–3.3

Abbreviations: See previous tables.

DQB1*0502. It was not associated with DR2 (DRB1*15, DRB1*16). Anti-dsDNA was associated with DRB1*15 (66.2 vs 52.2, p = 0.008, pc = NS, OR = 1.8, 95% CI = 1.2–2.7), DQA1*0102 (63.6 vs 50.4, p = 0.004, pc = NS, OR = 1.7, 95% CI = 1.2–2.5), and DQB1*0602 (66 vs 53.5, p = 0.03, pc = NS, OR = 1.7, 95% CI = 1.1–2.7).

Anti-Ro showed negative association with DRB1*01 (11 vs 27.6, p = 0.004, pc = NS, OR = 0.3, 95% CI = 0.1–0.7), DRB1*14 (3.6 vs 26.5, p = 0.01, pc = NS, OR = 0.1, 95% CI = 0.01–0.8), DQA1*0101 (10.2 vs 28.3, p = 6×10^{-4}, pc = 0.02, OR = 0.3, 95% CI = 0.1–0.6), DQB1*0501 (10.7 vs 28, p = 0.001, pc = 0.03, OR = 0.3, 95% CI = 0.1–0.6), and DQB1*0503 (0 vs 26.5, p = 0.006, pc = NS, OR = 0.7, 95% CI = 0.6–0.8). Anti-U1-RNP was negatively associated with DRB1*03 (12 vs 21.4, p = 0.01, pc = NS, OR = 0.5, 95% CI = 0.3–0.8), and DQA1*0501 (13 vs 24, p = 0.002, pc = NS, OR = 0.5, 95% CI = 0.3–0.7). Anti-nDNA was negatively associated with DQB1*0301 (48.2 vs 59.1, p = 0.04, pc = NS, OR = 0.6, 95% CI = 0.4–0.9). The other autoantibodies showed no association with HLA class II alleles.

Correlation of clinical manifestations with HLA class II alleles and with autoantibodies

Frequency of clinical manifestation was compared among the 11 centers. The following clinical manifestations showed a significantly different frequency: leukopenia (pc = 6×10^{-4}, ranging from 20% in patients coming from Austria to 80% in patients from Catalonia, Spain), lymphopenia (pc = 6×10^{-4}, ranging from 10% in patients from central/southern Italy to 85% in French patients), lymphadenopathy (pc = 6×10^{-4}, ranging from about 37% in French and Greek patients to about 20% in patients in all other centers), central nervous system involvement (pc = 6×10^{-4}, ranging from about 69% in patients from

France and Poland to 15% in those from northern Italy), livedo reticularis (pc = 6×10^{-4}, ranging from about 43% in patients from Poland and Greece to about 15% in those from the other centers). The association between these clinical manifestations and HLA alleles could be due to environmental factors or might be due to regional biases regarding diagnosis, and has to be considered cautiously. All other clinical manifestations showed a similar frequency across the 11 centers.

Some HLA class II alleles were associated with clinical manifestations of SLE (Table 6), perhaps due to their association with autoantibodies. Indeed, DRB1*03 was associated with pleuritis, lung involvement, renal involvement, and psychosis. DRB1*16 carried a higher risk for renal involvement, discoid lupus, and lymphadenopathy. DQA1*0101 was associated with lymphadenopathy, and DQA1*03 with lung involvement, lymphopenia, and hemolytic anemia. DQB1*0201 (DQw2) was associated with leukopenia, digital skin vasculitis, and pleuritis, while DQB1*0502 (DQw1) was associated with renal involvement, discoid lupus, and livedo reticularis.

Conversely, several HLA class II alleles were negatively associated with clinical manifestations of SLE (Table 7). DRB1*01 showed negative associations with renal and CNS involvement; DRB1*11 was negatively associated with renal disease and lymphopenia; and DRB1*14 was negatively associated with leukopenia, lymphopenia, and digital skin vasculitis. Concerning the DQ locus, DQA1*0101 showed negative associations with cardiac and renal disease, DQA1*0102 with CNS involvement, DQB1*0301 with arthritis and renal involvement, DQB1*0501 with renal involvement, and DQB1*0503 with leukopenia.

Some of these clinical manifestations were associated with the presence of autoantibodies. Namely, anti-Ro was associated with pleuritis (39.9 in patients anti-Ro positive vs 22.8 in those anti-Ro negative, p =

TABLE 6. Relative frequency of major clinical manifestations in patients positive and negative for the various HLA alleles (positive associations)

Clinical Manifestation	HLA	+	−	p Value	OR	95% CI
Pleuritis	DRB1*03	33.7	24.6	0.03	1.6	1–2.3
Lung involvement	DRB1*03	12.5	6.1	0.02	2.2	1.2–4.1
Renal involvement	DRB1*03	51.6	40.8	0.02	1.6	1.1–2.2
Psychosis	DRB1*03	8.1	3.5	0.04	2.4	1.1–5.3
Raynaud	DRB1*07	40.3	26.3	0.005	1.9	1.2–2.9
Renal involvement	DRB1*16	26.3	16	0.04	1.9	1.1–3.3
Discoid lupus	DRB1*16	25	12	0.004	2.4	1.3–4.4
Lymphadenopathy	DRB1*16	26.3	13.8	0.009	2.2	1.2–3.8
Lymphadenopathy	DQA1*0101	26.4	13.8	0.005	2.2	1.3–3.8
Raynaud	DQA1*0201	39.5	26.8	0.01	1.8	1.2–2.8
Lung involvement	DQA1*03	20	7.7	0.04	3	1.2–7.8
Lymphopenia	DQA1*03	73.3	44.7	0.004	3.4	1.5–7.8
Hemolytic anemia	DQA1*03	23.9	9.1	0.003	3.1	1.6–4.8
Leukopenia	DQB1*0201	53.9	44.5	0.04	1.5	1–2.1
Digital vasculitis	DQB1*0201	25.9	14.8	0.002	2	1.3–3.1
Pleuritis	DQB1*0201	32.5	23.7	0.03	1.6	1.1–2.3
Lung involvement	DQB1*0303	20	7.7	0.04	3	1.2–7.8
Lymphopenia	DQB1*0302	61.7	44.9	0.04	2	1.1–3.7
Lymphopenia	DQB1*0602	55.4	44	0.04	1.6	1–2.4
Renal involvement	DQB1*0502	30.8	19.4	0.02	1.8	1.1–3
Discoid lupus	DQB1*0502	24.5	11.6	0.002	2.5	1.4–4.3
Livedo reticularis	DQB1*0502	31.9	21.3	0.04	1.7	1–2.8

Abbreviations: See previous tables.

1×10^{-4}, OR = 2.2, 95% CI = 1.5–3-3), and with lung involvement (15.4 vs 5.2, p = 2×10^{-4}, OR = 3.3, 95% CI = 1.8–6.2). Anti-La was associated with pleuritis (44.4 vs 25.9, p = 0.03, OR = 2.3, 95% CI = 1.15–4.5), and showed negative association with renal disease (2.8 vs 18.4, p = 0.03, OR = 0.1, 95% CI = 0.02–0-9).

Although found in the context of SLE, other clinical manifestations that are more typical of the antiphospholipid syndrome are discussed in another paper (7).

Discussion

Multiple loci contribute to SLE, and disease pathogenesis is expressed as a complex genetic interaction that is undoubtedly influenced by environmental factors.

Genetic studies are complicated because the disease is polygenic, with susceptibility derived from a number of genes with relatively small effects (30), possibly interacting (22). Genetic studies in SLE are

TABLE 7. Relative frequency of major clinical manifestations in patients positive and negative for the various HLA alleles (negative associations)

Clinical Manifestation	HLA	+	−	p Value	OR	95% CI
Renal involvement	DRB1*01	10.5	23.3	0.02	0.4	0.2–0.8
CNS involvement	DRB1*01	1.3	8.6	0.05	0.1	0.01–0.9
Renal involvement	DRB1*11	28.3	43.9	0.005	0.5	0.3–0.8
Lymphopenia	DRB1*11	34.9	49.2	0.01	0.5	0.4–0.9
Leukopenia	DRB1*14	17.9	50.5	0.001	0.2	0.08–0.6
Lymphopenia	DRB1*14	2.5	47.5	0.03	0.4	0.01–0.9
Digital vasculitis	DRB1*14	3.6	20.8	0.05	0.1	0.02–0.9
Cardiac involvement	DQA1*0101	2.2	10.1	0.03	0.2	0.05–0.8
Renal involvement	DQA1*0101	13.2	23.2	0.05	0.5	0.3–0.9
CNS involvement	DQA1*0102	39.4	48.8	0.04	0.7	0.5–0.9
Arthritis	DQB1*0301	67.6	78.1	0.02	0.6	0.4–0.9
Renal involvement	DQB1*0301	33.8	48.5	0.003	0.5	0.4–0.8
Renal involvement	DQB1*0501	11.5	23.4	0.02	0.4	0.2–0.8
Leukopenia	DQB1*0503	15.4	50.5	0.001	0.2	0.06–0.5

Abbreviations: See previous tables.

**TABLE 8. HLA-DR, DQ associations with
SLE in different ethnic groups**

Ethnicity	No. of Patients	HLA Associations	Ref.
Netherlands	99	DRB1*03	28
Mexico (Mestizo)	58	DR3, DQA1*0501, DQB1*0201° DR1, DQA1*0101, DQB1*0501°	4
Spain	85	DR3 DQA1*0501	21
Greece	46	DRB1*1501, DRB1* 1601 and DRB1*0701	34

°Haplotype.

difficult also because SLE is heterogeneous, divisible in several clinical and immunologic subsets, each with its own genetic background. Furthermore, genes predisposing to SLE may vary according to the ethnic origin of the patient.

Studies, including association and family studies, have confirmed the linkage of the disease to the MHC region in humans (19). It also has been suggested that certain MHC haplotypes show stronger association with autoantibodies in SLE than with the disease itself (2).

Studies of the association between HLA class II alleles and SLE, and between HLA and serologic subtypes of the disease, were reviewed in depth by Reveille in 1999 (26), and we refer the reader to that paper for reference consultation. In Table 8 and Table 9 we have listed the most important associations described by other authors starting in 1998 (4, 6, 21, 25, 27, 28, 34). In summary, these studies found that HLA-DR3 was associated with Caucasian SLE patients in North America, the United Kingdom, Australia, Spain, Italy, Scandinavia, Switzerland, Austria, and, more recently, also in Germany (27) and the Netherlands (28), while in Greece the most significant association was with HLA-DR5 (DR11), and in northern India, with HLA-DR4. On the other hand the HLA-DR2 allele DRB1*1501 was associated with SLE in Caucasians and Asian groups including Japanese, Chinese, and Koreans, while in black patients the HLA-DR2 association was with the DRB1*1503 allele. In Mexican-Americans SLE was found to be associated with

HLA-DR3, and in both Mexican and Peruvian mestizos, with HLA-DR8. More recently (27) SLE was found to be associated with HLA-DQA1*0501 and DQB1*0201 in Caucasian patients and with HLA-DQB1*0602 in African Americans. In addition, in Mexican mestizo patients, an association has been found with HLA-DR3, DQA1*0501, DQB1*0201, and with HLA-DR1, DQA1*0101, and DQB1*0501 haplotypes (4). In Spanish patients an association of SLE with both HLA-DR3 and HLA-DQA1*0501 has been found (21), while in Greek SLE patients, associations with HLA-DRB1*1501, *1601, and *0701 have been described (34).

Concerning the HLA-DR and DQ genes and autoantibody subsets of SLE, associations (26) have been found between anti-dsDNA antibodies and DR2 (DRB1*1501) and DR3 and their respective linked HLA-DQ alleles (DQA1*0102, DQB1*0602 and DQA1*0501, DQB1*0201), as well as, in those lacking these alleles, DQB1*0302. An association of anti-dsDNA antibodies with HLA-DR15 and Dw2 in French Canadian SLE patients has been reported (25).

Anti-Ro and anti-La antibodies have been found to be associated with HLA-DR2 and DR3 and with the concomitant presence of DQ1 and DQ2. Furthermore, studies from 2 different European centers have implicated the HLA-B8-C4A*Q0-DRB1*0301-DQA1*0501-DQB1*0201 haplotype in susceptibility to anti-Ro/La autoantibody responses in Caucasians. In Japanese patients, the only association reported has been with HLA-DR8. The concomitant production of anti-Ro and anti-La antibodies has been found to be associated with HLA-DQA1*0501 and DQB1*0201 alleles in Spanish patients (21). More recently, an association has been found in Italy between anti-Ro/La response and HLA-DRB1*03011, DQA1*0501, and DQB1*0201 haplotype (6).

Anti-U1-RNP antibodies have been found to be associated in both Japanese and Caucasians with HLA-DR4 and DQw3 (the serologic group comprising HLA-DQB1*0301, *0302, and *0303). Subsequent studies, using oligotyping and DNA sequencing, have implicated both HLA-DRB1 and HLA-DQB1 alleles—specifically shared epitopes between HLA-DR2 and DR4 and HLA-DQB1*0302, linked to HLA-DR4—as

**TABLE 9. HLA class II alleles and autoantibody
subsets of SLE**

Autoantibody	Ethnicity	No. of Patients	HLA Associations	Ref.
Anti-ds DNA	Canada (French)	91	DR15 and Dw2	25
Anti-Ro/La	Spain	85	DQA1*0501 and DQB1*0201	21
Anti-Ro/La	Italy	undefined	DRB1*03011, DQA1*0501, DQB1*0201°	6

°Haplotype.

well as other specific DQB1 alleles sharing specific epitopes with DQB1*0302 in both Caucasians and Japanese.

All studies mentioned above do have limitations. The studies often did not use homogeneous clinical criteria for patient assessment, and they used different laboratory methods for the detection of autoantibodies that cannot be compared. In addition the number of patients recruited is usually rather low.

Because of the above-mentioned considerations, we wanted to verify the characteristics of the association of SLE with HLA class II alleles in a large sample of European patients. In fact, the large sample size allowed us to divide patients into various subsets, defined according to clinical characteristics and autoantibody status, and the common origin of the patients allowed us to overcome the problem of ethnic influence on HLA-SLE association. On the other hand, results of our study may be hampered at least in part by the relatively high number of participating centers, because we can not rule out completely the influence of environmental factors and of biases regarding diagnosis. The results presented here suggest that the overall contribution of the MHC to the disease itself is much less than its contribution to certain types of autoantibodies (and, in turn, to related clinical manifestations).

In fact, we have already shown that particular HLA class II alleles (DRB1*04, DRB1*07, DQA1*0201, DQA1*0301, DQB1*0302), in the context of SLE, predispose to the production of antiphospholipid antibodies and, as a consequence, to the development of clinical manifestations related to the antiphospholipid syndrome (9). We have also found significant association between other HLA alleles and both anti-ganglioside antibodies and related clinical manifestations (7).

In this paper, we show that, in the same patient sample, other HLA class II alleles confer susceptibility for different autoantibodies and possibly related clinical manifestations. For instance, patients carrying the DRB1*03 allele and the closely linked DQB1*0201 allele show genetic predisposition to the production of autoantibodies to Ro, La, and to pulmonary involvement, pleuritis, and psychosis. Interestingly, a similar association between DQB1*0201 and anti-Ro/La antibodies has been previously described in a Spanish population. To our knowledge, we have found for the first time that patients carrying the DQB1*0502 allele, which is in linkage disequilibrium with DR2, are prone to develop anti-Ro antibodies without anti-La antibodies, renal disease, discoid lupus, and livedo reticularis, whereas the presence of anti-La antibodies might be protective for renal disease. The association of Raynaud phenomenon and DRB1*07 (never described before, to our knowledge) is probably a consequence of the association of this allele with

antiphospholipid antibodies (9). DR2 (DRB1*15, DRB1*16) was associated with anti-dsDNA antibodies, confirming data previously described in Caucasian populations. It is noteworthy that we found for the first time, as far as we know, that DR2 haplotypes were also associated with renal disease, discoid lupus, and lymphadenopathy. DRB1*11 (DR5) appeared to protect against severe lupus, characterized by nephritis and lymphopenia, while SLE patients carrying DR1 showed a decreased risk for renal and CNS involvement, together with a decreased frequency of anti-Ro. Furthermore, DRB1*14 was associated with a decreased risk of having anti-Ro, and patients with this allele were less prone to develop digital skin vasculitis, leukopenia, and lymphopenia. The negative association of anti-dsDNA antibodies with DQB1*0301 could explain why patients with this allele had less renal disease.

Therefore, our results confirm that the role of HLA class II genes in SLE is to influence the production of the autoantibody repertoire and clinical manifestations, whereas the onset of SLE is more likely the consequence of the cooperation of many other non-HLA genes, interacting with appropriate external stimuli. Indeed, recent family studies showed that the genetic predisposition to SLE is only in minimal part located on the short arm of the sixth chromosome. Other regions of the human genome appear to be more important for SLE susceptibility, such as those located in chromosome 1, 2, 14, 16, and 20 (19). In addition, family studies show that different susceptibility loci are involved in the development of SLE in different ethnic populations, confirming the genetic heterogeneity of this disease.

In conclusion, our study of a large sample of Caucasian patients with lupus shows some new HLA clinical and serologic associations in SLE and reinforces the concept that this disease is genetically complex and that the role of MHC genes is mainly to predispose to particular serologic and clinical manifestations.

Summary

We conducted this study to determine the HLA class II allele associations in a large cohort of patients of homogeneous ethnic derivation with systemic lupus erythematosus (SLE). The large sample size allowed us to stratify patients according to their clinical and serologic characteristics. We studied 577 European Caucasian patients with SLE. Antinuclear antibodies (Hep-2 cells), anti-dsDNA antibodies (*Crithidia luciliae*), and antibodies to extractable nuclear antigens Ro (SS-A), La (SS-B), U1-RNP, Sm, Jo1, SCL70, and PCNA, were detected in all patients. Molecular typing of HLA-DRB1, DRB3, DQA1, and DQB1 loci was performed by the polymerase chain

reaction-sequence specific oligonucleotide probes (PCR-SSOP) method.

We found a significantly increased frequency of DRB1*03, DRB1*15, DRB1*16, DQA1*0102, DQB1*0502, DQB1*0602, DQB1*0201, DQB1*0303, and DQB1*0304 in lupus patients as compared with healthy controls. In addition, DRB1*03 was associated with anti-Ro, anti-La, pleuritis, and involvement of lung, kidney, and central nervous system. DRB1*15 and DQB1*0602 were associated with anti-dsDNA antibodies; DQB1*0201 with anti-Ro and anti-La, leukopenia, digital skin vasculitis, and pleuritis; and DQB1*0502 was associated with anti-Ro, renal involvement, discoid lupus, and livedo reticularis.

In conclusion, our study shows some new HLA clinical and serologic associations in SLE and further confirms that the role of MHC genes is mainly to predispose to particular serologic and clinical manifestations of this disease.

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