CONCISE REPORTS

HLA-DP does not contribute towards susceptibility to systemic lupus erythematosus

E J Davies, C J Hutchings, M C Hillarby, R P Donn, R G Cooper, E M Hay, R M Bernstein, P J L Holt, D M Grennan, W E R Ollier

Abstract

***Objectives*—To determine whether HLA-DP genes are involved in determining susceptibility to systemic lupus erythematosus (SLE).**

***Methods*—HLA-DPA1 and DPB1 genes were amplified by PCR of DNA samples from a panel of patients with SLE and normal controls. Amplified DNA was blotted on to nylon filters and probed with sequence-specific oligonucleotide (SSO) probes.**

***Results*—No DPA1 or DPB1 allele was significantly associated with SLE, or with any immunological or clinical subset of SLE. Evidence was found for only limited linkage disequilibrium between HLA-DP and HLA-DQ/DR variants, and none between HLA-DP and the TAP2 gene.**

***Conclusions*—These data indicate that HLA-DP genes do not contribute towards determining susceptibility to SLE.**

(*Ann Rheum Dis* 1994; 53: 188–190)

University of Manchester Rheumatic Diseases Centre, Hope Hospital, Salford, United Kingdom
E J Davies
M C Hillarby
R G Cooper
D M Grennan

ARC Epidemiology Research Unit, Stopford Building, University of Manchester, Manchester, United Kingdom
C J Hutchings
R P Donn
W E R Ollier

University of Manchester Rheumatism Research Centre, Manchester Royal Infirmary, Manchester, United Kingdom
E M Hay
R M Bernstein
P J L Holt

Correspondence to:
Dr E J Davies,
Rheumatic Diseases Centre,
Clinical Diseases Centre,
Clinical Sciences Building,
Hope Hospital,
Eccles Old Road,
Salford M6 8HD,
United Kingdom.

Accepted for publication
8 December 1993

Systemic lupus erythematosus (SLE) is a clinically heterogeneous connective tissue disorder characterised by immunological abnormalities including autoantibodies to double-stranded (ds) and single-stranded (ss) DNA and to various extractable nuclear antigens, such as Ro, La and U1-RNP. The role of these autoantibodies in the aetiopathogenesis of SLE is poorly understood.

There is a genetic predisposition to the disease demonstrated by a concordance of approximately 24% in monozygotic twins[1] compared with only 3% in dizygotic twins.[2] Part of this genetic predisposition is accounted for by genes within the MHC. There are well-documented associations of the class II antigens HLA-DR3,[3] DR2[4] and the class III phenotype C4A*Q0[5 6] with SLE.

Recent studies have found HLA-DQ genes to be more strongly associated with SLE than HLA-DR in certain populations,[7 8] suggesting that the main MHC susceptibility gene(s) for SLE may lie more centromeric than previously thought. We have shown that the TAP2 transporter gene, which lies centromeric to HLA-DQ but telomeric to HLA-DP, is not involved in SLE predisposition.[9] The limited linkage disequilibrium between TAP2 and DQ/DR suggests that any disease susceptibility gene centromeric to TAP2 is likely to constitute an independent risk factor for SLE.

Several studies have examined whether HLA-DP genes are involved in determining susceptibility to SLE. Some have found no DP association with SLE,[3 10] while others report DP associations either independent of [11] or secondary to [12] the DR associations. The role of HLA-DP in SLE therefore remains controversial.

We have examined the frequencies of HLA-DPA1 and HLA-DPB1 alleles in UK white patients with SLE to determine whether genes within the DP region are implicated in predisposition to SLE. We have also examined whether linkage disequilibrium exists between HLA-DP and other class II genes in this group of patients.

Materials and methods

SLE AND CONTROL POPULATIONS

White patients with SLE from north west England who fulfilled the ARA revised criteria for SLE[13] were used in this study. Patients were consecutive hospital attenders. Demographic, clinical and immunological details were recorded on a standard proforma. The median age of onset of SLE was 31 years, with a range of 10–78 years. The female:male ratio of the patient group was 9:1. The control samples used in the study consisted of local healthy volunteers.

DNA EXTRACTION

Genomic DNA was extracted from peripheral blood leucocytes as described previously.[14]

DPA AND DPB TYPING

A standard PCR sequence specific oligonucleotide (SSO) typing procedure was followed. 1–5 μg genomic DNA was amplified with DPA or DPB specific primers. Amplified PCR product (10 μl) was blotted on to positively charged nylon membrane (Boehringer Mannheim) using a vacuum dot blotter. Membranes were probed with DPB or DPA specific oligonucleotide probes from the British Society for Histocompatibility and Immunogenetics class II oligotyping kit. DPB1 oligonucleotide probes were labelled with digoxi-

Table 1 DPA1 and DPB1 allele frequencies (%) in SLE and controls

Allele	*SLE*	*Controls*
DPA1*	n = 86	n = 70
01	100	90·0
02	36·0	40·0
DPB1*	n = 77	n = 83
0101	26·0	16·9
0201	22·1	15·7
0202	1·3	0
0301	6·5[1]	19·3
0401	77·9	71·1
0402	27·3	26·5
0501	1·3	1·2
0601	1·3	6·0
0801	3·9	0
0901	0	1·2
1001	1·3	4·8
1101	0	7·2
1301	3·9	3·6
1401	0	2·4
1501	0	0
1601	0	1·2
1701	0	0
1801	3·9	1·2
1901	2·6	0

[1]p = 0·03 χ^2 = 4·66 p_c = NS OR = 0·29

p_c = correctedprobability (only calcualted if p < 0·05)
NS = not statistically significant

genin and detected with AMPPD according to the manufacturer's instructions (Boehringer Mannheim). DPA1 oligonucleotide probes were radiolabelled with ^{32}P by polynucleotide kinase. Probe stringency was tested using amplified homozygous HLA-DP cell line DNA.

DETECTION OF AUTOANTIBODIES

Detection and quantification of antibodies to double-stranded DNA was carried out by radioimmunoassay (Amersham Int). The presence of autoantibodies to four extractable nuclear antigens (Ro, La, RNP, Sm) was determined by immunodiffusion and counter-current immunoelectrophoresis.

Table 2 DPA1 and DPB1 allele frequencies (%) in subsets of SLE

Allele	*DNA*		*Ro + La*		*Ro*		*RNP*		*Renal*		*Vasc*	
	+	−	+	−	+	−	+	−	+	−	+	−
DPA1*												
n	57	29	16	70	11	75	14	72	23	63	11	75
01	100	100	100	100	100	100	100	100	100	100	100	100
02	35·1	37·9	56·3	31·4	18·2	38·7	42·9	36·1	34·8	36·5	27·3	37·3
DPB1*												
n	49	28	14	63	11	66	13	64	21	56	10	67
0101	24·5	28·6	35·7	23·8	18·2	27·3	23·1	26·6	33·3	23·2	20·0	26·9
0201	24·5	17·9	14·3	23·8	27·3	21·2	15·4	23·4	19·0	23·2	30·0	20·9
0202	0	3·6	0	1·6	0	1·5	0	1·6	0	1·8	0	1·5
0301	6·1	7·1	7·1	6·3	9·1	6·1	15·4	4·7	0	8·9	0	7·5
0401	81·6	71·4	78·6	77·8	81·8	77·3	76·9	78·1	71·4	80·4	100	74·6
0402	24·5	32·1	21·4	28·6	36·4	25·8	46·2	23·4	14·3	32·1	0	31·3
0501	0	3·6	7·1	0	0	1·5	0	1·6	0	1·8	0	1·5
0601	2·0	0	0	1·6	0	1·5	0	1·6	0	1·8	0	1·5
0801	4·1	3·6	7·1	3·2	0	4·5	0	4·7	9·5	1·8	10·0	1·5
1001	2·0	0	0	1·6	0	1·5	0	1·6	4·8	0	0	1·5
1301	2·0	7·1	0	4·8	0	4·5	0	4·7	4·8	3·6	0	4·5
1801	4·1	3·6	0	4·8	0	4·5	7·7	3·1	14·3[1]	0	0	4·5
1901	4·1	0	14·3[2]	0	0	3·0	0	3·1	4·8	1·8	10·0	1·5

[1]p = 0·02 χ^2 = 4·95 p_c = NS
[2]p = 0·03 χ^2 = 4·45 p_c = NS

For subsets of SLE, allele frequencies are compared with those SLE patients negative for that immunological/clinical feature.

DNA = circulating antibodies to dsDNA
Ro + La = circulating antibodies to both Ro and La
Ro = circulating antibodies to Ro alone
RNP = circulating antibodies to U1 RNP
Renal = renal disease
Vasc = vasculitis.

STATISTICAL METHODS

Chi-square analysis with Yates' correction was used to determine the significance of differences in allele frequencies between patients and controls. Levels of significance were corrected for multiple comparisons. Where appropriate, odds ratios (OR) were calculated. Linkage disequilibrium between HLA-DP and other class II alleles was assessed by calculating delta values (Δ). Chi-square analysis was used to determine the significance of linkage disequilibrium.

Results

The frequencies of HLA-DPA1 and DPB1 alleles in SLE and controls are shown in table 1. HLA-DPA1*01 was present in all patients with SLE and in 90% of control subjects. DPA*0201 frequency was non-significantly increased in patients with SLE with circulating antibodies to both Ro and La (table 2). No DPB1 allele was significantly associated with SLE or any subset of SLE. HLA-DPB1*0101 frequency was non-significantly increased in SLE (p = 0·16, OR = 1·73), particularly in patients with circulating anti-La antibodies. HLA-DPB1*0301 frequency was decreased in SLE (p = 0·03, p_c = NS, OR = 0·29). Both patients with SLE who were DPB1*1901 positive possessed antibodies to both Ro and La (p = 0·03, p_c = NS). All three patients possessing DPB1*1801 had renal disease (p = 0·02, p_c = NS). No DPA1 or DPB1 allele was found to occur at a significantly different frequency in those SLE patients with an earlier disease onset (30 years of age or below) compared with those patients with a later disease onset (after age 30 years).

Linkage disequilibrium analysis in patients with SLE found HLA-DPA1*02 to be associated with DPB1*0101 (Δ = 0·059) and negatively associated with DPB1*0401 (Δ = −0·091). Linkage disequilibrium between HLA-DP and HLA-DQ/DR was limited. HLA-DPB1*0101 was associated with DQA*0501 (Δ = 0·052) and DR5 (Δ = 0·029). HLA-DPB1*0201 was found to be in linkage disequilibrium with DQB*0603 (Δ = 0·036) and DR13 (Δ = 0·032). HLA-DPB1*0401 was negatively associated with DR3 (Δ = −0·11). HLA-DPB1*0402 was associated with DQA*0102 (Δ = 0·027) and DQB*0604 (Δ = 0·026). No HLA-DP alleles were found to be in linkage disequilibrium with any TAP2 allele in our patient population with SLE.

Discussion

In this study we have sought to determine whether genes in the HLA-DP region contribute to SLE susceptibility. No DPA1 or DPB1 allele was found to be associated with SLE. These results agree with the findings of other recent studies.[3 10]

We have also found no evidence for any DP allele being associated with immunological or clinical subsets of SLE. HLA-DPB1*0401 has been reported as being associated with the

presence of antibodies to U1-RNP[15] in connective tissue disease patients, independent of the DR4 association with this subgroup. HLA-DPB1*0301 and DPB1*1401 have increased in SLE, primarily in patients possessing anti-Sm/RNP and anticardiolipin antibodies.[11] We have been unable to confirm these associations in the present study and HLA-DPB1*0301 was found to be non-significantly decreased in our patient population with SLE. The failure to observe any DP associations with subgroups of SLE may, however, be due to the relatively small sizes of these groups in this study.

One recent study has reported a significant association of DPB1*0101 with SLE in a white population.[12] This association was found to be due to linkage disequilibrium of DPB1*0101 with the HLA-B8 DR3 haplotype. We have also found this allele to be more frequent in our patient population, albeit non-significantly. Linkage disequilibrium analysis shows DPB1*0101 to be associated with DPA1*02, DR5, DR3 (non-significantly) and DQA*0501 (data not shown). These findings are particularly interesting since DQA*0501 is found on both DR3 and DR5 bearing haplotypes and is the allele most strongly associated with SLE in our previous study.[8] Such linkage disequilibrium would explain the increased frequencies of DPA1*02 and DPB1*0101 in the anti-La antibody-positive subgroup of SLE, with these increases being secondary to the increased DQA*0501 frequency in this subgroup.[8] The limited associations detected between DP and DQ/DR explain why some associations of DP genes with disease are found to be independent of other class II associations while others are not.

Hence the HLA-DQ and DR associations with SLE we have previously reported do not reflect a stronger association with HLA-DP genes, and linkage disequilibrium between DP and DQ/DR is limited. This study provides no evidence that HLA-DP genes play a role in determining susceptibility to SLE.

We are grateful to the Arthritis and Rheumatism Council for Research and Lupus UK for financial support. E J Davies is funded by a Frederick Craven Moore award from the University of Manchester.

1 Deapen D, Escalante A, Weinrib L, *et al.* A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum* 1992; **35:** 311–8.
2 Winchester R J, Nunez-Roldan A. Some genetic aspects of systemic lupus erythematosus. *Arthritis Rheum* 1982; **25:** 833–7.
3 Reveille J D, Anderson K L, Schrohenloher R E, Acton R T, Barger B O. Restriction fragment length polymorphism analysis of HLA-DR, DQ, DP and C4 alleles in Caucasians with systemic lupus erythematosus. *J Rheumatol* 1991; **18:** 14–8.
4 Howard P F, Hochberg M C, Bias W B, Arnett F C, McLean R H. Relationship between C4 null alleles, HLA-D region antigens, and genetic susceptibility to systemic lupus erythematosus in caucasian and black Americans. *Am J Med* 1986; **81:** 187–93.
5 Fielder A H L, Walport M J, Batchelor J R, *et al.* Family study of the major histocompatibility complex in patients with systemic lupus erythematosus: importance of null alleles of C4A and C4B in determining disease susceptibility. *BMJ* 1983; **286:** 425–8.
6 Batchelor J R, Fielder A H L, Walport M J, *et al.* Family study of the major histocompatibility complex in HLA DR3 negative patients with systemic lupus erythematosus. *Clin Exp Immunol* 1987; **70:** 364–71.
7 Goldstein R, Sengar D P. Comparative studies of the major histocompatibility complex in French Canadian and non-French Canadian Caucasians with systemic lupus erythematosus. *Arthritis Rheum* 1993; **36:** 1121–7.
8 Davies E J, Hillarby M C, Cooper R G, *et al.* HLA-DQ, DR and complement C4 variants in SLE. *Br J Rheumatol* 1993; **32:** 870–5.
9 Davies E J, Donn R P, Hillarby M C, Grennan D M, Ollier W E R. Polymorphisms of the TAP2 transporter gene in systemic lupus erythematosus. *Ann Rheum Dis* 1994 (in press).
10 Reveille J D, Brady J, MacLoed-St. Clair M, Durban E. HLA-DPB1 alleles and autoantibody subsets in systemic lupus erythematosus, Sjogren's syndrome and progressive systemic sclerosis: a question of disease relevance. *Tissue Antigens* 1992; **40:** 45–8.
11 Galeazzi M, Sebastiani G D, Passiu G, *et al.* HLA-DP genotyping in patients with systemic lupus erythematosus: correlations with autoantibody subsets. *J Rheumatol* 1992; **19:** 42–6.
12 Yao Z, Hartung K, Deicher H G, *et al.* and Members of the SLE Study Group. DNA typing for HLA-DPB1 alleles in German patients with systemic lupus erythematosus using the polymerase chain reaction and DIG-ddUTP-labelled oligonucleotide probes. *Eur J Immunogen* 1993; **20:** 259–66.
13 Tan E M. Cohen A S, Fries J F, *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; **25:** 1271–7.
14 Kunkel L M, Smith K D, Boyer S H, *et al.* Analysis of human Y chromosome specific reiterated DNA in chromosome variants. *Proc Natl Acad Sci USA* 1977; **24:** 1245–9.
15 Hsu K-C, Hill DL, Hoffman RW. HLA-DPB1*0401 is associated with the presence of autoantibodies reactive with the U1-70 kD polypeptide antigen of U1-small nuclear ribonucleoprotein among connective tissue disease patients. *Tissue Antigens* 1992; **39:** 272–5.