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The Association of the HLA Class II Antigens with Clinical and Autoantibody Expression in Malaysian Chinese Patients with Systemic Lupus Erythematosus

M.R. Azizah[1], S.S. Ainol[2], S.H. Kuak[1], N.C.T. Kong[3], Y. Normaznah[1] and M.N. Rahim[4]

SUMMARY **The frequency of the HLA class II antigens/alleles (HLA-DR, DQ and DP) were studied in 70 Malaysian Chinese patients with systemic lupus erythematosus (SLE) to examine the contribution of these genes to disease susceptibility, their clinical expression and immunological responses. This was done using modified PCR-RFLP technique. These samples were then compared with 66 ethnically matched controls. We found a strong association of the DQA1*0102 (*p* corr = 0.032, rr = 3.39), DQB1*0501 (*p* corr = 0.003, rr = 4.55) , *0601 (*p* corr = 0.006, rr = 4.22) and DPB1* 0901(*p* corr = 0.02, rr = 4.58) with SLE. Clinically, we found a strong association of DR2 and DQA1*0301 with renal involvement and DQA1*0102 with alopecia. Immunologically, statistical analysis (Chi-square test) showed a strong association of DQA1*0102 with anti-Ro/La antibodies while DQA1*0301 was observed to be strongly associated with antibodies to ds DNA. DQA1*0102 was found more frequently in those with a later disease onset (30 years of age or above). From these data we suggest that the HLA class II genes play a role in conferring disease susceptibility and clinical and immunological expression.**

Genetic factors have been known to be involved in the etiology of SLE. This has been shown by results of family aggregation studies and the increased concordance of SLE among monozygotic versus dizygotic twins.[1,2] Among these genes, the major histocompatibility complex has proven to be the most consistent association with SLE. Numerous studies have been carried out on the association of the DR and the DQ alleles in SLE susceptibility among different ethnic/ racial groups. In the whites with SLE, an association has been found in DR3,[3,4] DR2 or both[5,6] while in the black Americans associations have been shown with DR3,[7] both DR2 and DR3.[8] Among the Chinese population reports of the strong association with DR2 have been observed,[9-11] while Savage *et al.*[12] did not find such association in the Singaporean Chinese. Previous studies have found DQ genes to be more strongly associated with SLE than DR in certain groups.[13,14] However, not many have studied the role of the DP locus as a predisposing gene in SLE; probably because of difficulties in typing by cellular methods. Aside from the fact that these genes may affect disease susceptibility, they are also known to affect clinical and autoantibody expression. Here, in this study we have examined the frequency of the HLA-DR, DQ and DP in our local Chinese SLE population to determine the role of these genes in disease susceptibility and their association in expressing certain clinical manifestations and immunological abnormalities.

MATERIALS AND METHODS

Patients

Seventy Chinese patients with SLE attending the SLE Clinic of the National University Hospital of Malaysia and who met the

From the [1]Biotechnology Centre, Institute for Medical Research, Kuala Lumpur, [2]Hospital Penang, Penang, [3]HUKM, Cheras, [4]Department of Bio-Medicine, Faculty of Allied Science, UKM, Malaysia.
Correspondence: M.R. Azizah

American College of Rheumatology (formerly American Rheumatism Association) classification criteria for SLE[15] were included into the study. There were 64 (91%) females and 6 (9%) males, with a female:male ratio of 10.6:1. Their mean age at study entry was 33 ± 12 years (mean ± SD), ranging from 15-69 years of age while the mean disease duration was 8 ± 6 years. Controls were taken from healthy unrelated blood donors with no history of any rheumatic disease. Medical history was taken from the case records and these features included: arthritis, malar rash, photosensitivity, oral ulcers, alopecia, renal involvement. According to the age of disease onset, two groups were established: early onset (below 30 years of age) and late onset (at 30 years of age or above). Laboratory features investigated were antibodies to the extractable nuclear antigens (Sm, RNP, Ro and La) and anti-ds DNA antibodies.

HLA typing

Genomic DNA was obtained from peripheral blood leucocytes using the salting-out technique.[16] DNA typing for "broad" DR groups (DR1-10) was determined by PCR while alleles of the DQA1, DQB1 and DPB1 genes were typed by a modified PCR-RFLP as previously described.[17,18] Genomic DNA was amplified by the PCR procedure with 2.5 units of the *Taq* DNA polymerase (Fermentas AB, Lithuania). The reaction mixture (100 µl), containing dNTPs (200 µM) and 2.5 mM $MgCl_2$, was subjected to 35 cycles of 1 minute at 96°C (denaturation), 1 minute at 55°C (annealing) and 2 minutes at 72°C (extension) by an automated PCR thermocycler (Perkin Elmer Cetus Inc) for the DQB1 gene. As for the DQA1 and DPB1 gene, the reaction mixture was subjected to 30 cycles of 1 minute denaturation at 94°C, 1 minute of annealing at 62°C and 2 minutes of extension at 72°C. After amplification, aliquots of the reaction mixture were digested by restriction endonucleases at optimum temperature after adding incubation buffer and distilled water. *Fok* 1, *Apa* 1, *Hae* II, *SfaN* I and *BssH* II, *Hph* I, *Bgl* I and *Sac* I, *Acy* I and *Hpa* II were used for digestion of the amplified DQB1 gene, *ApaL* I, *Hph* I, *BsaJ* I, *Fok* I, *Mbo* II and *Mnl* I for DQA1 and *Bsp*12861, *Fok* I, *Dde* I, *BsaJ* I, *BssH* II, *Sau96* I, *Rsa* I, *EcoN* I and *Ava* II for the amplified DPB1 gene. Electrophoresis was then performed and cleavage or no cleavage was detected by staining with ethidium bromide. Alleles were assigned by comparing RFLP patterns obtained with the relevant charts given.

Measurement of autoantibodies

Sera from patients were tested for the presence of antibodies to Sm, RNP, Ro and La and anti-ds DNA by using commercial ELISA kits (IMMCO Diagnostics, USA).

Statistical analysis

A 2 x 2 contingency analysis (x 2 test) with Yates correction or Fishers exact test where appropriate, was used to compare the MHC allele frequency in patients and controls. This was done with the EPI-INFO statistical program (Centers for Diseases Control, Atlanta, GA). *P* values of less than 0.05 were taken to be significant. *P* corr was determined by multiplying *p* value with the number of relevant comparisons made (HLA antigens/alleles). The strength of the association of HLA markers with SLE was measured by the relative risk and this was determined by the odds ratio. Statistical associations between the clinical and immunological findings and HLA antigens in patients with SLE (antibody positive patients with SLE, antibody negative patients with SLE) were determined by Fishers exact test.

Table 1 Clinical and immunological features of patients with SLE

Disease features	Frequency No. (%)
Age of disease onset	
< 30 years	19 (27%)
≥ 30 years	51 (73%)
Renal involvement	38 (54%)
Arthritis	30 (43%)
Malar rash	52 (74%)
Photosensitivity	43 (61%)
Oral ulcers	11 (16%)
Alopecia	41 (59%)
Anti-Sm/RNP	28 (40%)
Anti-Ro/La	40 (57%)
Anti-ds DNA	45 (64%)

Table 2 Frequency of HLA-DR antigens in Chinese SLE patients and healthy ethnically matched controls

DR	SLE patients N = 70 (%)	Controls N = 66 (%)	*p* value	*p* corr	rr
1	1 (1.4)	1 (1.5)	ns		0.94
2	58 (82.9)	50 (75.8)	ns		1.55
3	13 (18.6)	12 (18.2)	ns		1.03
4	13 (18.6)	9 (13.6)	ns		1.44
5	16 (22.9)	30 (45.5)	0.0053	0.053	0.36
6	8 (11.4)	12 (18.2)	ns		0.58
7	6 (8.6)	1 (1.5)	ns		6.09
8	5 (7.1)	6 (9.1)	ns		0.77
9	6 (8.6)	5 (7.6)	ns		1.14
10	1 (1.4)	8 (12.1)	0.02	0.2	0.11

ns = non-significant
p corr = *p* corrected
rr = relative risk

RESULTS

The clinical and immunological features of our patients with SLE are shown in Table 1. Most of these patients are in the late onset group. Clinically, among the mucocutaneous symptoms studied, the majority had malar rash (74%) while only 16% had oral ulcers. Sixty-four percent of patients were found to have antibodies to ds DNA. The frequency of the HLA-DR antigens, DQ and DP allele frequencies are shown in Tables 2, 3 and 4, respectively. When compared to the controls, we did not find any significant association of the HLA-DR with SLE. However,

Table 3 Frequencies of HLA-DQA1 and DQB1 alleles in Chinese patients with SLE and controls

HLA	SLE patients N = 70 (%)	Controls N = 66 (%)	*p* value	*p* corr	rr
DQA1					
0102	61 (43.6)	44 (33.3)	0.004	0.032*	3.39
0601	5 (7.1)	10 (15.2)	ns		0.43
0501	22 (31.4)	27 (40.9)	ns		0.66
0301	33 (47.1)	27 (40.9)	ns		1.29
0103	8 (11.4)	5 (7.6)	ns		1.57
0201	7 (10)	10 (15.2)	ns		0.62
0401	2 (2.9)	3 (4.5)	ns		0.62
0101	2 (2.9)	4 (6.1)	ns		0.46
DQB1					
0501	19 (27.1)	5 (7.6)	0.0027	0.003*	4.55
0502	9 (12.9)	18 (27.3)	0.035	0.42	0.39
0503	13 (18.6)	11 (16.7)	ns		1.14
0601	28 (40)	9 13.6)	0.0005	0.006*	4.22
0602	6 (8.6)	3 (4.5)	ns		1.97
0604	5 (7.1)	4 (6.1)	ns		1.19
0301	11 (15.7)	21 (31.8)	0.027	0.32	0.4
0302	24 (34.3)	36 (54.5)	0.017	0.2	0.43
0303	2 (2.9)	6 (9.1)	ns		0.29
0201	23 (32.9)	12 (18.2)	0.005	0.06	2.2
0401	0 (0)	5 (7.6)	0.02	0.24	0.00
0402	0 (0)	2 (3)	ns		0.00

*$p < 0.05$
ns = non-significant
p corr = *p* corrected
rr = relative risk

Table 4 HLA-DPB1 allele frequencies in Chinese SLE patients and healthy controls

DPB1	SLE patients N = 70 (%)	Controls N = 66 (%)	*p* value	*p* corr	rr
0101	14 (20)	10 (15.2)	ns		1.4
0201	7 (10)	12 (18.2)	ns		0.5
0202	0 (0)	0 (0)	ns		0.00
0301	5 (7.1)	4 (6.1)	ns		1.19
0401	27 (38.6)	19 (28.8)	ns		1.55
0402	12 (17.1)	14 (21.2)	ns		0.77
0501	2 (2.9)	4 (6.1)	ns		0.46
0601	0	0	ns		0.00
0801	2 (2.9)	10 (15.2)	ns		0.16
0901	22 (31.4)	6 (9.1)	0.0012	0.02*	4.58
1001	6 (8.6)	6 (9.1)	ns		0.94
1101	0 (0)	0 (0)	ns		0.00
1301	10 (14.3)	2 (3)	0.04	0.76	5.33
1401	7 (10)	6 (9.1)	ns		1.11
1501	0 (0)	0 (0)	ns		0.00
1601	1 (1.4)	2 (3)	ns		0.46
1701	1 (1.4)	3 (4.5)	ns		1.3
1801	1 (1.4)	0 (0)	ns		0.00
1901	0 (0)	2 (3)	ns		0.00

*$p < 0.05$
ns = non-significant
p corr = *p* corrected
rr = relative risk

HLA-DR5 and DR10 were found to be slightly decreased among the SLE patients as compared to controls but *p* corr was not significant. There was a significant increased frequency of HLA-DQA1*0102 (*p* corr = 0.032). Frequency of DQB1*0501 and *0601 was also significantly increased among patients. As for the HLA-DPB1, *0901 showed significant increase in the patient group. No other significant findings were noted.

HLA association with clinical manifestations

The association between genetic factors and clinical and autoantibody expression were evaluated in patients with SLE. Table 5 shows the association of the HLA antigens/alleles with clinical manifestations. In our samples, we found a significant increase of both DR2 and DQA1*0301 (*p* corr = 0.04, rr = 9.4 and *p* corr = 0.024, rr = 4.38, respectively) in patients with renal involvement compared with those without renal complications. HLA- DQB1*0501 was not significantly increased (*p* = 0.02, uncorr). There was a weak decrease of DQA1*0301 in the group of patients with arthritis as compared to those in the negative subgroup. However, when comparing patients with malar rash and those without, there was a non-significant decrease of DR3. We found a weak excess of DR5 (non-significant) in the subgroup of patients without photosensitivity as compared to those with photosensitivity. We did not observe any significant DR, DQ or DP specificity among those with oral ulcers. However, when comparison was made between patients with and without alopecia, DQA1-*0102 was found to be significantly associated with this symptom with a weak excess of DQA1*0103 in those without alopecia. DQA1-*0102 was strongly increased in patients of late onset disease while patients with early onset disease more commonly (though non-significant) exhibited HLA-DQA1-*0501 and *0103.

Table 5 HLA-DR, DQ and DP allelic frequencies (%) in Chinese SLE patients divided according to their clinical manifestations and age of onset (number of alleles in parentheses)

HLA	SLE (n = 70)	Renal (n = 38)	Arthritis (n = 30)	Malar rash (n = 52)	Photosensitivity (n = 43)	Ulcer (n = 11)	Alopecia (n = 41)	Onset (< 30 yrs) (n = 19)
DR								
2	83	95*	83	81	81	73	78	79
3	19	16	17	10	12	9	15	16
4	19	21	13	23	21	27	20	16
5	23	21	20	23	21	27	20	32
DQA1								
0102	44	95	97	94	93	91	92*	58*
0501	31	24	37	27	28	27	22	53
0301	47	63*	30	48	49	46	46	26
0103	11	11	17	8	9	9	2	26
DQB1								
0501	27	40	30	25	23	18	24	26
0502	13	18	10	15	14	36	20	5
0601	40	29	47	35	37	18	37	53
0301	16	13	17	17	19	9	12	11
0302	34	37	27	37	40	55	29	32
0201	33	42	23	29	26	55	31	47
DPB1								
0101	20	26	23	19	19	18	22	5
0402	17	18	17	15	14	36	15	26
0901	31	24	37	27	33	36	34	21
1301	14	21	10	17	19	9	17	21

*significant *vs* symptom negative patients

HLA association with immunological abnormalities

Table 6 shows the association of the HLA class II antigens/alleles with immunological abnormalities. There was no DR, DQ and DP specificity with anti-Sm/RNP antibodies. There was also no DR specificity with anti-Ro/La and anti-ds DNA antibodies. Comparing patients with anti-Ro/La antibodies and those without, we observed a strong association with DQA1*0102 (p corr = 0.0032, rr = 14.18) but there was a weak decrease of DQA1*0301 and DQB1*0501 in patients with the Ro/La antibodies. Both anti-Ro alone and anti-La alone were found to be significantly associated with DQA1*0102 (a finding which was similar to those with the anti-Ro/La subgroup) and DQB1*0601 (data not shown). This clearly shows the close association of the Ro and La antibodies with SLE. Comparing between anti-ds DNA antibody positive and negative patients, a significant decrease of HLA-DQA1*0301 was found in the antibody positive group (p corr = 0.032, rr = 0.19) with a weak decrease of DR4 and a weak increase of DQB1*0402.

DISCUSSION

In this study we have evaluated the role of HLA class II antigens and alleles in contributing to the predisposition of SLE. We further determined their association with clinical and immunological expressions. Though several studies among the Asian Chinese SLE population have found an association with the DR2 antigen,[9-11] we, however, could not confirm this. Savage *et al.*[12] in his study on the Singaporean Chinese SLE population did not find any DR specificity with SLE. Our study looked at the DQ locus (DQA1*0102 and DQB1*0501 and *0601) in conferring disease susceptibility. Our results raise the possibility that the DQ may have a direct effect on increasing susceptibility to SLE. Several investigators have also found a strong association between the DQ alleles and SLE. Beside an association with DR3, Skarvsvag *et al.*[19] also found a link with the DQ haplotype (DQA1*0501, DQB1-*0201) in his patients. Among Mexican Americans, the most common association was with the DQ2 haplotypes.[20] The role of the DPB

Table 6 HLA DR, DQ and DP allelic frequencies (%) in Chinese SLE patients divided according to their immunological abnormalities (number of alleles in parentheses)

HLA	SLE (n = 70)	Sm/RNP+ (n = 28)	Ro/La+ (n = 40)	DNA+ (n = 45)
DR				
2	83	79	83	84
3	19	25	25	13
4	19	18	10	9
5	23	21	25	27
DQA1				
0102	44	89	98*	93
0501	31	32	30	33
0301	47	50	35	33*
0103	11	11	13	9
DQB1				
0501	27	21	15	20
0502	13	7	15	20
0601	40	46	48	38
0301	16	11	18	18
0302	34	29	28	31
0201	33	39	30	27
DPB1				
0101	20	21	18	24
0402	17	14	20	24
0901	31	36	35	29
1301	14	14	15	9

*significant *vs* antibody negative patients

in disease susceptibility to SLE was controversial. Some have found no DP association with SLE[4,21] while others reported DP association either independent of[22] or secondary to the DR association.[23] In our study the DP allele, DPB1*0901 was strongly associated with the disease.

Many studies have tried to determine a particular HLA phenotype which might be strongly linked to a certain clinical manifestation but without success. We found some positive and negative associations of the HLA antigens with clinical disease manifestation in our group of SLE patients but these variabilities were not able to confirm those found by others. This could be due to the clinical heterogeneity of the patients enrolled in the studies and may also reflect the influence of ethnic/racial differences.

Here, we found that HLA-DR2 and DQA1*0301 were significantly increased in patients with renal involvement. The strong association with DR2 was in agreement with that found by others.[24,25] However, Hong *et al.*[9] found a significant decrease of DR2 while Ahearn *et al.*[26] did not find any HLA specificities that were associated with renal involvement. We also found weak associations of DQB1*0201 and DR5 with renal disease but these did not remain significant after multiplying by the number of comparisons made and thus could have probably occurred by chance.

While we did not find any HLA association with arthritis, Schur *et al.*[27] noticed a correlation with HLA-DQ1 with a low frequency of DR5 but no association was found by Hashimoto *et al.*[28] An association of malar rash with DPB1*0201 was also noted by Hashimoto *et al.*[28] There was an absence of an HLA association with arthritis, malar rash, photosensitivity and oral ulcers. Hashimoto *et al.*[28] found photosensitivity to be associated with DR4 and/or DQB1-*0401. While they found an association of DPA1*0201 with alopecia, in our study the association was with DQA1*0102. Unlike the finding of an association of DPB1*0901 with oral ulcers in his study,[28] we did not observe this HLA association.

Our finding of an association of DQA1*0102 in patients in the later age of disease onset group was different from that noticed by others.[14,29] While the HLA-B8 and DR3 were significantly increased in the later disease onset (above 35 years) in one study,[29] another instead found that DQA1*0501, and DR3 were elevated in patients with onset before 30 years of age.[14]

Since there are known close similarities between the Ro and the La antigens and between the Sm and the RNP antigens,[30,31] we have chosen to distinguish between antibody responses to these 2 different non-cross reactive nucleoprotein families. We did not find any HLA association with anti-Sm/RNP antibodies though DR2 or DR4 have been found to be associated with either anti-Sm or RNP antibody.[32,33] Our finding was supported by others.[26,34] In Japanese patients, DQ was associated with anti-RNP antibodies.[35] A study on the black population noticed a

strong association of anti-Sm with DR2[36] while another noted an association with DR7.[27]

The strong association of anti-Ro/La antibodies with the DQ allele (DQA1*0102) in this study suggests that the DQ role is more primary than the DR type. However, both DQA1*0102 and DQB1-*0601 alleles were significantly associated with anti-Ro alone or anti-La alone. Bell and Maddison[34] first reported a high frequency of DR3 (and B8) in white anti-Ro positive patients with SLE. This association in white patients was confirmed by others[26,32,37] along with DQ2 linked to DR3. Unlike those mentioned earlier, Hochberg *et al.*[38] found no increased HLA specificities, including DR3, DR2 and DR7 and anti-Ro in Baltimore blacks with SLE. Nishikai and Sekiguchi[35] could correlate no DR or DQ antigens with anti-Ro in Japanese patients. Hamilton *et al.*[39] found DR2 significantly associated with anti-Ro without anti-La and DR3 with anti-Ro and anti-La. Reveille *et al.*[40] observed DR, DQ and DP alleles in white and black patients with anti-Ro antibodies and found DR3, DQw2.1 and heterozygosity for DQ6/DQ2.1 as the strongest association. Anti-Ro in Mexican Americans was found to be strongly associated with DR3, DQA1*0501, DQB1*0201 haplotype.[20] DR3 negative, anti-Ro positive patients most frequently had DR2, DQA1*0102, DQB1*0602 haplotype. In Greeks with SLE,[41] no specific HLA alleles or haplotypes were found to correlate with anti-Ro and anti-La antibodies. Anti-DNA antibodies showed a strong association with DQA1-*0301 in this study. However, in other studies high levels of anti-ds DNA were found to be associated with DR2 and possibly DQ1.[26,37] Others have found the association with DR3[42] and DR7[27] while another noticed a strong association with the DQB1 alleles (*0201, *0602 and *0302).[43]

In summary, several studies on different ethnic groups have found strong correlation of SLE with the class II HLA specificities. Most findings have noted susceptibility genes to be at the DR locus (DR2, DR3 or both, DR4 or DR7), and/or DQ and DP in some studies. Though variations in the literature occur in the genes involved in disease susceptibility and their linkage with clinical manifestations and autoantibody responses, these could be due to racial differences. We suggest that the HLA system plays a role in conferring disease susceptibility. However, this may not be the primary risk factor in clinical and immunological disease expression but exhibits epistatic or modifying effects.

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