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The genetic basis of Ro and La antibody formation in systemic lupus erythematosus

Results of a multicenter study

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Summary. Antibodies against Ro and La, including recombinant La and recombinant 60 kD-Ro, were determined by counter immunoelectrophoresis and ELISA in over 300 central European systemic lupus erythematosus (SLE) patients. The presence of both Ro and La antibodies was strongly associated with the MHC haplotype B8-C4AQ0-DR3-DQ2, the association being strongest for DR3. After exclusion of all B8-DR3 positive patients only DR3 positive patients still showed an increased incidence of Ro and La antibodies, suggesting DR3 as the primary association factor. High titers of La antibody, but not of 60 kD-Ro antibody, were also significantly associated with the presence of DR3. Other DR and DQ antigens or heterozygous DQ combinations were not significantly associated with Ro and La antibodies.

Key words: Systemic lupus erythematosus – Ro and La antibodies – Multicenter study – Genetics

Introduction

Various genetic factors have been implicated in the pathogenesis of systemic lupus erythematosus (SLE). An increased concordance has been observed in identical twins, a high incidence of SLE has been observed among individuals with certain inborn complement deficiencies and certain MHC gene products have been reported to be associated with the disease [1–4]. Thus, various clinical and serological features of the disease have been found to be associated with the presence of certain HLA antigens [5–8], especially the formation of autoantibodies against Ro and La [9–11], ssDNA and dsDNA [10–13], Sm and nRNP [8, 10, 11, 14], other nuclear antigens [15], and cardiolipin [16–18].

Ro and La antibodies, which appear in up to 15% (La) and 60% (Ro) of SLE patients [19, 20], have been proposed to be markers for certain clinical subsets of lupus, e.g. lupus with complete C2-deficiency, subacute cutaneous lupus erythematosus, neonatal lupus and ANA-negative lupus [20–26]. Various associations of Ro and La antibodies with HLA antigens, complement alleles [22, 27], T-cell receptor genes [28], and Gm-allotypes [29] have also been described in SLE: While the majority of authors have found an association of Ro and La antibodies with HLA-B8 and HLA-DR3 in SLE and other diseases [9–11, 30], some have reported associations of Ro antibody with DR2, DR6, and DQ1 [13, 31–35] that were not observed by others [10, 14, 26, 33, 36]. Recently two groups have suggested that two different genetic MHC backgrounds [DR3 (La), and DQ1/DQ2-heterozygosity (Ro)] may not only confer susceptibility to the appearance of Ro and La in SLE, but also influence the titers of Ro antibody [36, 37].

As part of a central European multicentric study on SLE currently under way, we investigated MHC associacions of Ro and La antibodies measured by different methods in 376 SLE patients and 402 first degree relatives and spouses.

Patients and methods

We examined 376 Caucasian patients with SLE from a central European multicenter study. All patients fulfilled the revised ARA criteria for the classification of SLE [38]. Patients with overlap syndromes were carefully excluded from the study. All patients were recruited from the out-patient clinics of the University Clinics at Hannover, Düsseldorf, Freiburg and Erlangen, Germany, between 1987 and 1990.

HLA typing. HLA of HLA-B, DR and DQ antigens was performed using a standard complement-dependent microcytotoxicity assay [39]. Frequency data for HLA-B, -DR and -DQ antigen distributions in European Caucasians from the HLA Workshop 1984 were

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used as controls [40]. Correlations with antibodies were sought for the HLA-B antigen B8, DR1 to 10 and DQ1 to 3, as well as DR2/3-heterozygotes, presumed DR3-homozygotes (DR3/X-phenotype) and DQ combinations, as well as C4Q0 alleles.

Electrophoretic characterization of C4. C4 electrophoretic variants were determined in neuraminidase-treated ethylenediaminetetraacetate (EDTA) plasma samples using the technique of immunofixation electrophoresis in agarose gels as previously described [41]. When necessary, a distinction between C4A and C4B locus products was achieved by immunoblotting using a monoclonal antibody specific for C4B locus products [42].

The assignment of phenotypes to individuals showing low levels of C4 protein on test gels was confirmed by testing two independent plasma samples. The assignment of the null alleles AQ0 and BQ0 was made on the basis of differential staining intensities of the electrophoretically separated C4A and C4B products. Within a certain protein range, binding of Coomassie blue is proportional to the concentration of protein [43]; therefore, a binding pattern of C4A of twice the intensity of C4B indicates that the individual tested is C4B heterozygous, carrying C4BQ0. Individuals heterozygous for both AQ0 and BQ0 need to be identified by family segregation analysis. The nomenclature for alleles of the two C4 loci was adopted from the 4th International Complement Workshop, Boston, USA [44].

ENA determination. Ro and La antibodies were determined according to standard procedures [45] by counter immunoelectrophoresis (CIE) with spleen extract (Zeus Scientific Inc., USA) and calf thymus extract (Pel-Freeze, Ark., USA) as substrates. For ELISA testing, recombinant 60 kD-Ro [46] and recombinant La [47] proteins were obtained by PCR cloning of cDNA derived from Hep-G2 (La) or Jurkat (Ro) cell mRNA and expression in *E. coli* according to standard procedures [48]. Briefly, after oligo dT-primer directed reverse transcription of Hep-G2 or Jurkat cell mRNA, Ro and La cDNAs were amplified using suitable oligonucleotide primers complementary to each 5'- and 3'-end of the encoded 60 kD-Ro or La regions which additionally provided EcoRI or BamHI restriction sites at their 5'-ends. Amplified products of the expected sizes (Ro 1610 bp, La 1220 bp) were digested with BamHI and EcoRI, purified by preparative gel electrophoresis and directionally ligated into the similarly treated expression vector pEx34b [49]. In-frame ligation of amplification products was confirmed by dideoxy sequencing. Transformed *E. coli* 537 colonies were selected by size analyses of Ro or La insert DNAs, each of the five colonies bearing Ro or La inserts of the correct size were grown. Protein synthesis controlled by the inducable lambda-promoter was achieved by increasing the temperature to 42 °C. The Ro-MS-2 polymerase fusion protein was purified by extraction with 10 M urea, followed by separation of SDS PAGE; the La-MS-2 polymerase fusion protein was purified by extraction with 7 M urea, followed by separation on heparin sepharose CL6B and DEAE sepharose. Before and after final purification, the sizes and purity of the fusion proteins were demonstrated by Coomassie blue staining on SDS gels, immunoreactivity of the fusion proteins was confirmed by immunoblotting using well characterized own reference sera and reference sera of CDC, Atlanta, USA. The recombinant fusion proteins were applied to ELISA plates at concentrations of 2–3 µg/ml. Patients' sera were analyzed at 1 : 300 dilutions, calculations of results were performed using standard curves prepared from two well characterized sera of known Ro or La specificity. We assigned 400 U/ml of anti-60 kD-Ro or anti-La activity to the 1 : 300 dilutions of these sera. With these standard curves, the mean antibody activities of sera from 15 healthy donors were 8.9 ± 2.4 U/ml (anti-La) or 9.5 ± 3.1 U/ml (anti-Ro). La antibodies were regarded as positive if greater than 40 U/ml, recombinant 60 kD-Ro antibodies were regarded as positive if greater than 25 U/ml, and recombinant La antibodies were regarded as positive if greater than 25 U/ml. For statistical analysis, the chi-square and Wilcoxon-Mann-Whitney rank sum tests were employed.

Results

Ro antibodies were detected in 114 out of 360 patients (32%), and anti-La in 48 out of 360 patients (13%) as determined by CIE. Antibodies against recombinant 60 kD Ro were present in 60 out of 375 patients (16%). A highly significant correlation was found between anti-60 kD-Ro and anti-Ro as determined by CIE ($\chi = 117$, $P < 10^{-8}$); individuals positive for anti-60 kD Ro formed a subgroup of those displaying Ro antibodies in their serum (Fig. 1 a). Anti-La as determined by ELISA were present in 93 out of 376 patients (25%). As expected from results of similar studies [19, 20], the anti-La-ELISA proved to be more sensitive than anti-La-CIE, with a highly significant correlation between the two tests ($\chi = 119$, $P < 10^{-8}$; Fig. 1 b).

The correlation between the ELISA results with La and recombinant La protein antigens was also highly positive ($\chi = 77$, $P < 10^{-8}$), with the recombinant La-ELISA detecting the respective La antibody in 67 out of 364 of the SLE sera (18%). The overwhelming majority (79%) of anti-La positive sera as determined by CIE was also positive for Ro antibody ($\chi = 57$, $P < 10^{-8}$; Fig. 1 c).

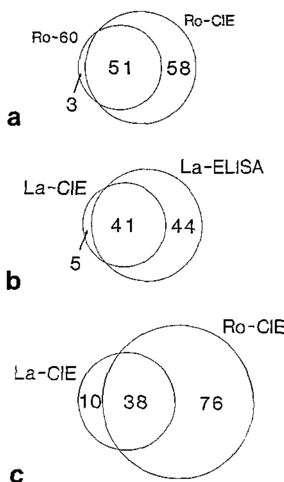

Fig. 1 a–c. Correlations between Ro and La antibody tests. Absolute numbers of SLE patients positive for the respective tests are presented.
a Anti-Ro as determined by counterimmunoelectrophoresis (Ro-CIE) and anti-recombinant 60 kD-Ro (ELISA) ($n = 346$ patients tested in both tests).
b Anti-La as determined by counterimmunoelectrophoresis (La-CIE) and anti-La (ELISA) ($n = 347$ patients tested in both tests).
c Ro and La antibodies by CIE ($n = 360$ patients tested in both tests)

Table 1. Antibodies to Ro and La in spouses and relatives of SLE patients, and percentage of DR3 in positive tests. DQ1/2 was present in only five of 32 relatives with positive ENA tests, with differing DR specificities: DR1/3, 1/3, 2/3, 2/7 and 6/7. There was also no association of antibodies with DR2. rec La = recombinant La

	Test positive (%)	DR3 positive (%)	Frequency of HLA-DR3 in antibody-negative individuals (%)
Ro-CIE	4/254 (1.6)	2/4 (50)	32*
La-CIE	3/254 (1.2)	1/3 (33)	32
60 kD-Ro	7/388 (1.8)	2/7 (29)	32
La-ELISA	9/388 (2.3)	2/9 (22)	32
rec La-ELISA	17/388 (4.4)	8/17 (47)	32
Any Ro assay	11/402 (2.7)	4/11 (36)	32
Any La assay	24/402 (6.0)	10/24 (42)	32
Any Ro or La assay	32/402 (8.0)	12/32 (38)	32

* All $P > 0.01$

Of the total of 402 first degree relatives and spouses, less than 5% were positive for Ro and La antibodies in each of the five tests performed (Table 1).

Immunogenetic results

Both anti-Ro and anti-La antibodies as measured by CIE (Ro-CIE, La-CIE) showed a highly significant association with the presence of HLA-DR3 in SLE patients (Table 2). There was no significant association of both antibodies with any other DR specificity including DR6 and DR2. The incidence of DR2 was slightly lower in anti-La-positive individuals.

Table 2. Associations of HLA antigens and C4AQ0 alleles with Ro and La antibodies as determined by CIE in 311 SLE patients. pos = positive; neg = negative; hom = homozygous. *P*-values given are uncorrected; *P*-values in brackets are >0.05 after Bonferoni's correction for the number of tests performed ($n=21$)

HLA-phenotype/ C4 allele	Anti-Ro			Anti-La		
	pos (%)	neg (%)	$P<$	pos (%)	neg (%)	$P<$
DR1	10	14		13	13	
DR2	44	43		25	46	(0.02)
DR3	65	40	5×10^{-5}	80	44	2×10^{-5}
DR4	9	18		17	15	
DR5	11	18		8	17	
DR6	20	20		20	20	
DR7	16	21		10	21	
DR2/3	19	7		13	11	
DR3-hom	10	7		15	7	
B8	59	36	(0.0045)	73	39	3×10^{-5}
DQ1	70	70		58	72	
DQ2	77	57	(0.004)	85	59	0.002
DQ3	22	39	(0.004)	30	34	
DQ1/2	46	30	(0.004)	45	33	
DQ1/3	9	21	(0.02)	8	19	
DQ2/4	10	15		20	13	
DQ1-hom	14	19		5	20	(0.03)
DQ2-hom	18	13		20	13	
DQ3-hom	2	3		3	3	
C4AQ0	69	43	2×10^{-5}	83	46	3×10^{-6}
C4BQ0	11	23	(0.01)	8	21	(0.05)

La-CIE was also significantly associated with HLA-B8, DQ2 and C4AQ0 alleles, all of which are in strong linkage disequilibrium with DR3 on one haplotype in Caucasians [50]. C4AQ0 also showed a significant association with the presence of Ro antibody, and both HLA-B8 and -DQ2 were increased in Ro-CIE-positive patients. The increased frequency of DQ2 in anti-Ro and anti-La positive probands is, however, due solely to the linkage disequilibrium between DR3 and DQ2, as DR7 is not increased in antibody-positive patients (Table 2).

The significant immunogenetic associations observed for the antibodies against La (La-ELISA), recombinant La and recombinant 60 kD-Ro are displayed in Table 3. Similar to the CIE results, DR3 was significantly associated with anti-60 kD-Ro and anti-recombinant La, and was increased in La-ELISA-positive patients. C4AQ0 alleles, again, showed a significant association with the presence of all three antibodies; HLA-B8 and -DQ2 were not significantly increased among individuals positive for these antibodies. No other HLA-Dr or -DQ antigens tested showed any significant associations with the three ELISA tests (data not shown). Among the relatives and spouses of SLE patients those rare probands with Ro or La antibodies, or both, displayed no significant associations with HLA-DR3 (Table 1) or any other DR or DQ antigen (data not shown), although DR3 was slightly more frequent in antibody-positive relatives.

We then looked for immunogenetic differences between patients positive for both the Ro and La antibody (La^+Ro^+) in one of the test systems (CIE or ELISA), and those possessing only Ro antibodies. As depicted in Table 4, La^+Ro^+ probands displayed an increased frequency of HLA-DR3, -B8, -DQ2 and C4AQ0 alleles as compared to La^-Ro^+ ones. Ro positive individuals without La antibodies (La^-Ro^+) showed an insignificantly increased frequency of DR3, B8 and C4AQ0 as compared to patients negative for both antibodies (La^-Ro^-). In La^-Ro^+ patients a slight but insignificant increase of DR2 (55% versus 44%) was observed. The slightly increased frequencies of DR2/3- and DQ1/2-heterozygotes of La^-Ro^+ compared to antibody-negative ps proved to be insignificant after correction of the *P*-values for the number of tests performed. After exclusion of all DR3-positive patients, an association of DQ1/2 positivity with

Table 3. Associations of HLA antigens and C4Q0 alleles with Ro and La antibodies as determined by ELISA in 320 SLE patients. rec La = recombinant La; pos = positive; neg = negative. *P*-values given are uncorrected; *P*-values in brackets are >0.05 after Bonferoni's correction for the number of tests performed ($n=21$); all other HLA antigens: no significant differences

HLA-phenotype C4 allele	60 kD Ro (ELISA)			La (ELISA)			rec La (ELISA)		
	pos (%)	neg (%)	$P<$	pos (%)	neg (%)	$P<$	pos (%)	neg (%)	$P<$
DR2/3	20	9	(0.006)	11	10		5	11	
DQ1/2	46	32		42	32		34	33	
DR2	43	42		38	44		31	45	(0.05)
DQ1	70	70		69	70		61	71	
DR3	69	45	0.002	58	46		64	44	(0.004)
DQ2	74	60		69	60		73	59	(0.05)
B8	62	41	(0.005)	56	40	(0.009)	58	40	(0.009)
C4AQ0	71	47	0.0008	67	46	0.0003	70	46	0.0004

Table 4. Associations of HLA antigens and C4Q0 alleles with Ro^{+}/La^{-} and Ro^{+}/La^{+} antibody status in SLE patients. hom = homozygous. Patients positive for La in ELISA or CIE were regarded as La positive, patients positive for Ro in CIE or 60 kD-Ro-ELISA were regarded as Ro positive. *P*-values given are uncorrected; *P*-values in brackets are >0.05 after Bonferoni's correction for the number of tests performed ($n=20$). *P1* refers to differences between $La^{+}Ro^{+}$ and $La^{-}Ro^{+}$; *P2* refers to differences between $La^{-}Ro^{+}$ and $La^{-}Ro^{-}$

MHC-phenotype	$La^{+}Ro^{+}$ $n=30$	$La^{-}Ro^{+}$ $n=68$	$La^{-}Ro^{-}$ $n=203$	*P1**	*P2***
DR1	13	9	14		
DR2	23*	53	44	(0.007)	
DR3	87*	56	39**	(0.004)	(0.02)
DR4	10	9	17		
DR5	10	12	19		
DR6	23	19	20		
DR7	7	21	21		
DR2/3	17	21	7**		(0.003)
DR3-hom	17	7	6		
B8	79*	50	36**	(0.004)	(0.03)
DQ1	60	75	71		
DQ2	90*	67	56	(0.02)	
DQ3	23	21	38**		(0.01)
DQ1/2	53	43	30**		(0.05)
DQ1/3	3	12	21		
DQ2/3	17	8	14		
DQ1-hom	3	19	20		
DQ2-hom	20	16	12		
DQ3-hom	3	2	3		
	$n=38$	$n=73$	$n=228$		
C4AQ0	87*	58	43**	0.002	(0.03)

* *P1* (refers to differences between $La^{+}Ro^{+}$ and $La^{-}Ro^{+}$)
** *P2* (refers to differences between $La^{-}Ro^{+}$ and $La^{-}Ro^{-}$)

either $La^{+}Ro^{+}$ (versus $La^{-}Ro^{+}$) or with Ro^{+} (versus Ro^{-}) antibody status (both *P* values >0.2; data not shown) was no longer demonstrable.

When ELISA titers of the Ro and La antibodies were analyzed, a significant association of high anti-La titers with DR3 and a weak association of high anti-La titers with DQ2 were observed (Table 5). Antibody titers against the recombinant 60 kD-Ro antigen did not show any HLA-linked differences.

Finally, we investigated possible roles of single class I, II and III MHC components of the extended haplotype B8-C4AQ0-DR3-DQ2 in the formation of Ro and La antibodies. Considering that HLA-B8 and -DR3 occur in strong linkage disequilibrium it can be assumed that practically every Caucasian phenotypically positive for B8 and 83 actually carries this haplotype [40, 50]. Thus, segregation analysis in a family study comprising 174 families of SLE patients revealed that all spouses and patients phenotypically positive for B8 and DR3 possessed this haplotype [51]. As shown in Table 6, the haplotype B8-DR3 was found to be strongly associated with both antibodies among 129 patients both B8 and DR3 positive. On the contrary, among the remaining 192 patients, including those with B8, DQ2, and C4AQ0 without DR3, associations with Ro or La antibodies were not observed except for a marginal result for La^{+} individuals (CIE) ($P=0.04$). However, it may be noted that the relative frequencies of antibody positive and negative probands among B8-DR3 positives and DR3 positives (without B8) were quite similar (e.g. 43% vs 39% Ro positives and 24% vs 21% Ro negatives), and that the *P* values for "DR3 only" positive individuals were mar-

Table 5. Associations of ELISA antibody titers against La, recombinant La and recombinant 60 kD-Ro. rec La = recombinant La; pos = positive; neg = negative; hom = homozygous; ns = not significant. Numbers represent rank of antibody titers in HLA antigen positive and negative patients; in Wilcoxon-Mann-Whitney rank sum test; *P*-values given are uncorrected; *P*-values in brackets are >0.05 after Bonferoni's correction for the number of tests performed ($N=16$)

HLA-	La ($n=79$)			rec La ($n=64$)			Ro-60 ($n=51$)		
	pos	neg	*P*	pos	neg	*P*	pos	neg	*P*
DR1	47	39	ns	37	32	ns	32	25	ns
DR2	33	45	(0.025)	27	35	ns	23	29	ns
DR3	49	27	10^{-4}	36	26	(0.05)	28	22	ns
DR4	24	42	(0.03)	27	33	ns	18	27	ns
DR5	40	40	ns	33	33	ns	25	26	ns
DR6	43	39	ns	38	31	ns	36	24	ns
DR7	24	43	(0.008)	20	35	(0.03)	19	27	ns
DQ1	37	43	ns	31	32	ns	26	25	ns
DQ2	44	29	(0.006)	33	29	ns	26	24	ns
DQ3	35	41	ns	28	33	ns	23	27	ns
DQ1/2	41	38	ns	33	31	ns	26	25	ns
DQ1/3	28	40	ns	23	33	ns	26	25	ns
DQ2/3	46	38	ns	33	31	ns	21	26	ns
DQ1-hom	32	41	ns	35	31	ns	24	26	ns
DQ2-hom	50	37	ns	32	31	ns	30	25	ns
DQ3-hom	11[a]	40	(0.03)	–	–	–	8[b]	26	ns

[a] $n=3$
[b] $n=1$

Table 6. Associations of the haplotype B8-DR3 and its components with Ro and La antibodies in SLE. Numbers represent % of patients with the respective phenotype who are positive or negative for the antibody

Phenotype	Number of individuals	Ro (CIE)			La (CIE)			rec Ro (ELISA)			La (ELISA)			rec La (ELISA)		
		+	−	*P*	+	−	*P*	+	−	*P*	+	−	*P*	+	−	*P*
B8-DR3	$n=129$	43	24	0.0003	23	6	10^{-4}	23	12	0.009	30	21	ns	28	16	0.009
DR3 (without B8)	$n=28$	39	21	(0.04)	14	5	(0.07)	22	10	(0.06)	25	20	ns	25	14	ns
B8 (without DR3)	$n=17$	24	24	ns	0	7	ns	12	12	ns	24	21	ns	12	16	ns
AQ0 (without DR3)	$n=28$	15	25	ns	0	8	ns	4	13	ns	32	19	ns	14	16	ns
DQ2 (without DR3)	$n=41$	21	25	ns	5	7	ns	5	13	ns	17	22	ns	10	17	ns

ginal because of the low absolute frequency of such patients ($n=28$). Therefore, we may conclude that HLA-DR3 itself, or a very closely linked gene, may be associated with the presence of Ro and La antibodies.

Discussion

Anti-Ro and -La antibodies recognize protein epitopes on small ribonucleoproteins [52]. Four different antigenic Ro proteins of 60 kD, 52 kD and 54 kD have been isolated from human lymphocytes and erythrocytes; antibodies against the two 60 kD-proteins as well as antibodies against the 52 kD- and 54 kD-proteins show a high degree of crossreactivity [53]. Ben-Chetrit and coworkers have recently found in a smaller sample of patients, that 52 kD-antibodies are observed mainly in Sjögren's syndrome, while 60 kD-antibodies appear predominantly in SLE [54].

Our finding that the recombinant anti-60 kD ELISA recognized a subgroup of approximately 50% of all Ro antibodies as detected by CIE may be explained by the possibility that the recombinant Ro protein exhibits conformational epitopes different from the native molecule. The possibility of unspecific binding due to lack of purity of Ro antigens is less likely, because anti-Ro was detected by CIE in 32%, which is in keeping with the results of other authors [19, 20]. Furthermore, different antigenic specificities of anti-Ro antibodies, which were not detectable by the 60 kD-Ro ELISA, such as 52 kD-antigens, cannot be excluded. The low prevalence of antibody-positives found in relatives with all assay systems points to the fact that all assay systems are sufficiently specific [35]. The La polypeptide is complexed to the Ro ribonucleoprotein, and the majority of individuals positive for La antibodies also have Ro antibodies.

For SLE and other autoimmune diseases various associations of the presence of Ro and La antibodies with HLA antigens, namely B8, DR2, DR3, DR6, DR7, DQ1, DQ2, and DQ1/2-heterozygosity, have been reported with somewhat conflicting results [8–10, 13, 14, 30–37]. Our findings, in the largest sample of SLE patients examined so far, confirmed some previous reports, which state that both Ro and La antibodies are associated with HLA-DR3 in white SLE patients [8–10, 14]. We also observed that the antibody response against both the recombinant La and the 60 kD-Ro peptide was associated with DR3. To our knowledge this is the first report to show that the antibody response against recombinant Ro and La proteins, which are chemically unequivocally defined, is MHC-associated.

We also found Ro and La antibodies to be associated with HLA-B8, -DQ2 and C4AQ0-alleles. This was not an unexpected finding, because these MHC markers occur in strong linkage disequilibrium with DR3 on one extended haplotype B8-C4AQ0-DR3-DQ2 which is found with a frequency of about 7.5% in the white population [40, 50]. This haplotype is present in about 20% of white SLE patients [51]. We wondered if one of these four linked genes may be responsible for the association with Ro and La antibodies or may be most closely linked to the responsible gene. By discriminating between patients with the haplotype B8-DR3 and those possessing DR3, B8, DQ2 or C4AQ0 on other haplotypes we obtained evidence that DR3 or a gene closely linked to the DR locus is responsible for the association. Thus, patients carrying DR3 with haplotypes other than B8-C4AQ0-DR3-DQ2 showed an increased frequency of Ro and La antibodies nearly identical to that of the patients with the B8-DR3 haplotype. Generally, the association with DR3 was strongest in patients with La antibodies, but was also present in patients with anti-Ro without La antibodies. Furthermore, DR3 was associated with high titers of La antibodies. Whether the association of the Ro and La antibody response with HLA-DR3 is based on a general immunological hyperresponsiveness of individuals with the haplotype B8-DR3, as observed in allergic diseases, autoimmune diseases and in certain experiments by various investigators [55, 56] remains to be investigated. However, such an assumed hyperresponsiveness does not apply to other antibody systems such as Sm, nRNP and cardiolipin antibodies [8, 11, 13, 15–18]. HLA-DR3 may, therefore, exert a more specific effect on Ro and La antibody formation, e.g. by facilitated presentation of the Ro/La antigen to lymphocytes. In this context it would be rewarding to study population samples which differ from central and northern European Caucasians with regard to the prevalence of the haplotype B8-DR3, e.g. a Spanish sample, with a high incidence of the haplotype B18-DR3.

Contrary to some recent reports [13, 32–37] we did not find any significant associations of antibodies against Ro and La with DR or DQ specificities other than DR3 and DQ2. The frequencies of DR2, DR6, DR7 and DQ1

did not differ significantly between antibody-positive and -negative groups. We also did not observe a significant increase of DQ1/DQ2-heterozygotes in the group of patients with anti-Ro without anti-La, as has been postulated by two groups of investigators recently [34, 36, 37]. Hamilton et al. [34] have obtained their significant differences by comparing the frequencies of DR2 and DQ1 of anti-Ro-positive patients with those of a normal control population, but not with the frequencies of the antibody-negative patients (as would have been correct, because HLA-DR2 itself occurs with increased frequency in SLE, irrespective of other factors) [2, 10, 11]. The fact that Hamilton et al. have observed higher Ro antibody titers in DQw1/DQw2-positive patients in the Ro^+La^- patients can be explained by a coincidental clustering of DQw1 with DQw2 (i.e. also DR3) in a small number ($n=8$) of patients, with DR3 being the HLA marker primarily associated with high antibody titers.

Our final conclusion is, that in white SLE patients the antibody formation against both Ro and La, including recombinant 60 kD-Ro and recombinant La antigens, is influenced by the presence of HLA-DR3 (or a gene in strong linkage disequilibrium with DR3, such as DQ2.1) while no other DR and DQ antigens or heterozygous combinations are involved in this antibody response. Overall, the association with DR3 is stronger for La antibodies than for Ro antibodies.

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