MAJOR HISTOCOMPATIBILITY COMPLEX GENES AND SUSCEPTIBILITY TO SYSTEMIC LUPUS ERYTHEMATOSUS

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Susceptibility to systemic lupus erythematosus is associated with major histocompatibility complex (MHC)–encoded genes. We have used nucleotide sequence analysis to better define the disease-associated MHC alleles. HLA–DR2, DQw1, and especially the rare allele DQβ1.AZH confer high relative risk (RR = 14) for lupus nephritis in a Caucasian population of patients. Pilot studies using historical controls suggest that these genes also confer a high risk in non-Caucasian ethnic groups (RR = 24–78). We have found that DR4 is significantly decreased in patients with lupus nephritis. Fifty percent of the patients with lupus nephritis had either the DQβ1.1, the DQβ1.AZH, or the DQβ1.9 alleles. These alleles share amino acid residues that have been predicted to be the contact points for antigen and the T cell receptor. These HLA alleles appear to have a direct role in the predisposition to lupus nephritis, whereas DR4 may have a "protective" effect.

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder that involves the skin, joints, serosal surface, kidneys, central nervous system, and blood elements. It is characterized by abnormalities in B cell activation, with resultant autoantibody production, and by dysregulation of T cells, the complement cascade, and the clearing of immune complexes. Genetic factors in SLE have been implicated by results of studies of family aggregation of the disease (1), increased concordance of SLE among monozygotic versus dizygotic twins (2), Gm markers (3), decreased red cell CR1 receptors (4), abnormal T cell suppressor function in healthy relatives of SLE patients (5), and associations with several major histocompatibility complex (MHC) loci (summarized in refs. 6 and 7).

Population studies have shown an increased association between SLE and class II MHC DR2 and/or DR3 antigen alleles, as well as an association with class III MHC C2 and C4A deficiencies (8,9). The strengths of these associations vary from study to study, and they can vary in ethnically different populations (7–10). The relative risk (RR) of SLE in a person positive for any 1 of these markers has been reported to be 3 or less. However, the RR is significantly increased if multiple alleles are present, such as homozygosity of C4A null (RR = 16) or C4A null and DR2 (RR = 25) (9), which supports the notion that susceptibility to this disease may be polygenic.

The class II loci of the human major histocompatibility complex encode the HLA-D cell-surface

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glycoproteins, which are expressed on B lymphocytes, activated T lymphocytes, macrophages, and dendritic cells. These proteins, designated DR, DQ, and DP, consist of a variably polymorphic α subunit and a highly polymorphic β subunit. They are responsible for the presentation of foreign antigen to T cells. The genetic variability is localized to the NH_2-terminal extracellular domain, which is thought to interact with the T cell receptor and with peptide fragments of antigen(s) (11). The polymorphism of the HLA-D region products has conventionally been defined by serologic typing reagents (for DR and DQw) and by T cell recognition in mixed lymphocyte culture reactions in response to stimulation with homozygous typing cells (for Dw specificity) (12,13).

Class III gene products are involved in the clearance of immune complexes and the activation of inflammation-mediating molecules (14). Traditionally, factor B and the second and fourth complement components (BF, C2, and C4A and C4B) have been detected by protein electrophoresis (15–17). Such molecular techniques as restriction fragment length polymorphism (RFLP) using MHC locus–specific complementary DNA (cDNA) probes, sequence-specific oligonucleotide (SSO) analysis, and direct sequencing of the polymorphic first domain have refined the analysis of class II allelic polymorphism as well as the identification of C4A and C4B gene deletions (18–29).

Because of their potential pathogenetic role, the molecules encoded by the class II and class III MHC genes may have a direct role in predisposition to SLE. Our examination of these genes and their products by protein analysis, nucleotide sequencing, and nucleotide SSO probing has revealed that the DQβ allele 1.AZH, which is associated with the DR2(DRw16), DwAZH(Dw21),DQw1(DQw5) haplotype (updated HLA nomenclature [30] is shown in parentheses) confers a significant risk for lupus nephritis. DR4 was significantly decreased in SLE patients with nephritis, compared with its frequency in SLE patients without kidney disease and compared with normal control subjects. A novel DQβ allele (DQβ1.SLE) and an uncommon DQβ1.9 allele, both associated with DRw6,DQw1, were overrepresented among our Caucasian lupus patients (31,32). C4A null was detected in one-third of all the lupus patients; in two-thirds of these patients, this was due to a DR3-associated C4A gene deletion. The remaining one-third of the C4A-null patients had an absent protein product and a normal RFLP pattern, possibly suggesting a DR2 and DQw1–associated regulatory defect in C4A expression.

Our data suggest that the clinical subset of SLE patients who have nephritis is genetically more homogeneous, even between different ethnic groups. The DQβ alleles 1.AZH, DQβ1.1, and DQβ1.9, which share polymorphic residues in the first and third hypervariable regions (HVR), are overrepresented in our lupus nephritis population and may play a direct role in the predisposition to SLE nephritis. In contrast, DR4 may have a protective effect for this clinical subset. Aside from a potential direct role, these DQw1 susceptibility alleles may be linked to an MHC regulatory gene (such as tumor necrosis factor), which may independently predispose a person to the development of SLE.

PATIENTS AND METHODS

Patient and control populations. Patients seen at the clinics and hospitals of Stanford University Medical Center, University of California at Los Angeles, and University of California at San Francisco from 1985 through 1988 were entered into the study if they fulfilled the following criteria: 1) had SLE diagnosed according to the American Rheumatism Association criteria (33), 2) had evidence of SLE nephritis documented either by renal biopsy or the presence of proteinuria, hematuria, or renal failure, which was not due to another independent cause, or 3) had no renal disease and had been diagnosed as having SLE for at least 5 years (the risk of acquiring nephritis being the greatest in the first 5 years after SLE diagnosis).

Ethnic identity was determined by direct questioning. Of the 118 lupus patients studied, 78 were Caucasian, 21 were black, 10 were Asian, 7 were Filipino, 1 was Hawaiian, and 1 was a native of Guam.

The HLA class II typing controls for the Caucasian SLE patients were 91 normal Caucasian laboratory personnel from the Stanford Medical Center. Because the numbers of non-Caucasian controls available at Stanford were too small, we used the HLA-DR and DQw antigen frequencies obtained for North American blacks from the Eighth HLA Workshop (34). For the Asian, Polynesian, and Filipino populations, we used the frequencies from the third Asia-Oceania Histocompatibility Workshop and Conference (35) and from the Ninth HLA Workshop (36). For controls for the complotype analyses, we used 1,920 normal Caucasian haplotypes identified in 960 healthy subjects from the Boston area. A complotype is the cluster of the 4 complement genes within the class III MHC that are inherited as a single unit.

After obtaining informed consent from each study subject, 10–20 ml of venous blood was collected from each of the subjects. Peripheral blood lymphocytes (PBL) were separated and transformed with Epstein-Barr virus (EBV). The cell lines were serologically typed by the Stanford Blood Bank for HLA-DR and DQ, using standard microlymphocytotoxicity tests (37). Plasma was subjected to electrophoresis to type for BF, C2, and C4, as described previously (15–17).

RFLP analysis. DNA was extracted from the EBV-transformed cell lines or from PBL using standard techniques. Genomic DNA (5–10 μg) was digested with *Eco* RI, *Bam* HI, *Pst* I, *Pvu* II, *Taq* I, *Bgl* II, *Hind* III, and/or *Sst* I, according to the manufacturer's specifications, subjected to electrophoresis, and blotted in the conventional manner (38,39) onto Hybond-N membranes (Amersham, Arlington Heights, IL). Each filter was hybridized overnight at 42°C to approximately 50 ng of probe DNA that had been labeled by hexamer priming (40), washed at high stringency (55–65°C), and then exposed to Kodak XAR film. Full-length cDNA of the DRβ and DQβ genes obtained from cDNA clones from the same DR1-homozygous typing cell LG2 were used for DRβ and DQβ probes (41,42). A full-length cDNA clone obtained from the DR3 haplotype (Bell JI: unpublished observations) was utilized as the DQα probe. Full-length C2 and factor B cDNA probes (43) were generously provided by R. D. Campbell (Medical Research Council Immunochemistry Unit, Oxford, England). The C4 cDNA (29) probe was kindly provided by K. T. Belt (Pharmacia, Cambridge, England).

Sequence analysis. Sequence-specific oligonucleotide analysis and the cloning and sequencing of the DQβ first domain were performed using the polymerase chain reaction (PCR) technique (44). Genomic DNA was amplified with *Thermus aquaticus* (45) or with DNA polymerase I Klenow fragment. One-tenth of the purified PCR product was directly ligated into M13 sequencing vector and transformed into *Escherichia coli* JM101. Approximately 80% of these plaques contained class II sequences, and single-stranded DNA templates purified from the plaques were dideoxy-sequenced directly. One-fifth of the PCR product was blotted onto nitrocellulose paper and probed with radiolabeled detection SSO. The specific priming oligonucleotides, detection SSO, and methodologic details are described in detail elsewhere (27,32).

Statistical analysis. Fisher's exact test was used for most analyses (46). In cases of very large sample sizes, Woolf's formula for chi-square was employed, and Haldane's modification of Woolf's formula was used in sets containing 0 (47).

Nomenclature. The Ninth HLA Workshop terminology (48–50) is used in this report for 2 reasons. First, the serotypings were performed during a time when the Ninth HLA Workshop typing reagents were available. Second, although more detailed than prior workshops, the Tenth HLA Workshop nomenclature (30) still underestimates the complexity of the HLA loci. For example, using the nomenclature of the Tenth HLA Workshop, DQw5 specificity includes both DR1,DQβ1.1 and DR2,DQβ1.AZH. The DQw6 specificity includes 3 different DQβ alleles: DQβ1.2, 1.12, and 1.18. Because of the potential confusion from renaming subsets within the most recent nomenclature, we

Table 1. Frequency (%) of HLA–DR, DQβ alleles in systemic lupus erythematosus (SLE) patients with and without nephritis*

DR	DQβ	SLE with nephritis (n = 69)	SLE without nephritis (n = 36)	All SLE patients (n = 118)	Lupus nephritis patients vs. patients without nephritis	
					RR	*P*
1	1.1	18.8	16.7	18.6		
2†	1.2, 1.12, or 1.AZH	59.4	36.1	50.8	2.6	0.01
	1.2	39.1	33.0	36.4		
	1.AZH	24.6	5.6	17.8	5.6	0.01
3	2	30.4	25.0	26.3		
4	3.1 or 3.2	14.5	33.0	21.2	0.34	0.02
5	3.1	14.5	22.2	16.1		
w6	1.9, 1.18, 1.19, or 1.SLE	18.8	11.1	16.9		
	1.9	6.3‡	0§	3.6		
	1.18	4.7‡	2.8§	4.5		
	1.19	6.3‡	8.3§	8.0		
	1.SLE	1.6‡	2.8§	1.8		
7	2 or 3.1	14.5	13.9	16.1		
w8	4(blank)	1.4	5.6	2.5		
9	3.2 or 3.3	1.4	5.6	2.5		
DR1, 2, or w6	DQw1	87.0	55.6	75.4	5.3	0.0004
Shared amino acid residues						
26–30	1.2 and 1.SLE	39.1	38.9	38.1		
52–57	1.1, 1.19, and 1.SLE	23.2	22.2	24.6		
26–30 and 66–75	1.1, 1.AZH, and 1.9	46.4	22.2	38.1	3.0	0.009

* Thirteen patients had had SLE for <5 years; their data were included only in the total SLE patient group. DR was determined serologically; DQβ was determined by restriction fragment length polymorphism, sequence-specific oligonucleotide (SSO) analysis, and/or direct sequencing (see Patients and Methods for details). RR = relative risk; *P* values calculated by Fisher's exact test (46).

† The sum of the DR2-associated DQβ alleles is greater than the DR2 antigen frequency because several patients were heterozygous for DQβ1.2, 1.AZH.

‡ Corrected values. SSO or direct sequencing was done on only 12 of the 13 DRw6-positive patients with lupus nephritis.

§ The sum of these subtypes is >11.1% because 3 patients had 4 alleles (1 DRw6-positive SLE patient without nephritis was heterozygous for DQβ1.19, 1.SLE).

use modified Ninth Workshop terminology already in use in the literature (23,26,27,31). In our terminology, the DQβ gene from the DR1,Dw1(Dw20),DQw1(DQw5) haplotype is named DQβ1.1; the DQβ from the DR2(DRw16), DwAZH(Dw21),DQw1(DQw5) haplotype is named DQβ1.AZH; the DQβ from the DR2(DRw15),Dw2,DQw1 (DQw6) haplotype is named DQβ1.2; the DQβ from DR2 (DRw15),Dw12,DQw1(DQw6) haplotype is named DQβ1.12, and so forth.

RESULTS

Class II genes. RFLP analysis of SLE patient DNA with DRβ and DQα probes revealed normal patterns (data not shown). Analysis of *Eco* RI–digested DNA from patients with lupus nephritis probed with DQβ revealed an increased frequency of an uncommon DR2-associated DQβ subtype (51). This DR2,DwAZH,DQw1,DQβ RFLP was confirmed by sequencing the DQβ1.AZH first domain from several patients (32) and by SSO analysis of the remaining SLE patients' DNA after PCR amplification.

Table 1 summarizes the class II allele frequencies in our SLE population. DR2 was present in 59.4% of the 69 patients with lupus nephritis, compared with 36.1% of the 36 SLE patients without kidney disease (RR = 2.6, P = 0.01). Because the likelihood of developing renal disease is the greatest in the first 5

Table 2. Frequency (%) of HLA–DR, DQβ alleles in Caucasian SLE patients with and without nephritis and in Caucasian control subjects*

DR	DQβ	SLE with nephritis (n = 44)	SLE without nephritis (n = 25)	All SLE patients (n = 78)	Control subjects (n = 91)	Lupus nephritis patients vs. controls	
						RR	P
1	1.1	25.0	24.0	25.6	11.0	2.7	0.02
2†	1.2, 1.12, or 1.AZH	47.7	40.0	46.2	20.0	3.7	0.0007
	1.2	38.6	36.0	37.2	18.7	2.7	0.008
	1.AZH	13.6	8.0	12.8	1.0‡	14.2	0.005
3	2	31.8	20.0	25.6	20.0		
4	3.1 or 3.2	15.9	40.0	23.1	42.0	0.25	0.001
5	3.1	11.4	16.0	12.8	23.0		
w6	1.9, 1.18, 1.19, or 1.SLE	23.0	8.0	20.5	20.0		
	1.9	5.1§	0.0	2.6	2.5§		
	1.18	5.1§	0.0	3.8	12.4§		
	1.19	10.1§	8.0¶	10.3	4.9§		
	1.SLE	2.5§	4.0¶	2.6	0.0#		
7	2 or 3.1	18.2	12.0	16.7	25.0		
w8	4(blank)	2.3	0.0	1.3	5.0		
9	3.2 or 3.3	0.0	8.0	2.6	2.0		
w10	1.1	0.0	0.0	0.0	3.0		
1, 2, w6, or w10	DQw1	84.1	64.0	78.2	50.5	4.7	0.0003
Shared first and third HVR 1, 2, or w6	1.1, 1.AZH, or 1.9	50.0	32.0	45.0	16.3	5.1	0.00006
Shared first HVR 2 or w6	1.2 or 1.SLE	38.6	36.0	38.0	18.7	2.7	0.008
Shared second HVR 1 or w6	1.1, 1.19, or 1.SLE	34.0	36.0	36.7	16.7	2.6	0.01

* Nine patients had had SLE for <5 years; their data were included only in the total SLE patient group. HVR = hypervariable region. See Table 1 and Patients and Methods for details of DR and DQβ determinations, explanations of statistical calculations, and explanations of other abbreviations.

† The sum of the DR2-associated DQβ alleles is greater than the DR2 antigen frequency because several patients were heterozygous for DQβ1.2,1.AZH.

‡ Although none of the 12 DR2-positive controls examined by SSO were DQβ1.AZH-positive, this calculation was made assuming approximately 1% haplotype frequency for DwAZH in the normal Caucasian population (12,48–50). If DQβ1.AZH is 0 in the control population, the RR = 30.9, as calculated using Haldane's modification of Woolf's formula for sets containing 0 (47). An additional 6 DR2-positive controls were found to be DQβ1.2-positive, as analyzed by restriction fragment length polymorphism alone; this assignment is considered to be reliable because the DQβ allele on the second chromosome was not 1.2, 1.18, or 1.19 in any of these 6 controls, and DQβ1.AZH was therefore not masked (see Discussion for details).

§ Corrected values. SSO or direct sequencing was done on only 10 of the 11 DRw6-positive patients with lupus nephritis and only 8 of the 18 DRw6-positive controls.

¶ The sum of these subtypes is >8.0% because 3 patients had 4 alleles (1 DRw6-positive SLE patient without nephritis was heterozygous for DQβ1.19, 1.SLE).

DQβ1.SLE has not been detected in the 12 DRw6-positive Caucasian controls or lines tested by us (31,32) or the 42 DRw6-positive Caucasian subjects tested by other investigators (26,28).

years, patients free of renal disease but diagnosed as having SLE for fewer than 5 years were excluded from the nonrenal SLE patient subset; therefore, the total number of patients studied (n = 118) is greater than the sum of the renal and nonrenal SLE patient subsets (n = 105).

The rare DQβ allele 1.AZH was significantly increased in the renal disease group compared with that in the nonrenal group (24.6% versus 5.6%; RR = 5.6, $P = 0.01$). DQβ1.2 was increased overall, but the difference between the 2 subgroups was not significant. The sum of lupus patients typed by SSO as the DR2-associated DQβ subtypes 1.2, 1.12, and 1.AZH is greater than the total number of patients reported as DR2 because of 1.2, 1.AZH heterozygosity in 3 patients.

DR4 was significantly decreased in the renal disease patients (14.5% versus 33.0%; RR = 0.34, $P = 0.02$). Distribution of the DR4-associated DQβ3.2 and 3.1 was approximately 2 to 1 in both clinical groups and was similar to that in the healthy normal population. The increase in DRw6 among the lupus nephritis patients was not statistically significant.

DQw1 (associated with DR1, 2, and w6) was present in 87.0% of the lupus nephritis patients and in 55.6% of those without nephritis (RR = 5.3, $P = 0.0004$). The first and third HVR common to the DQβ1.1, DQβ1.AZH, DQβ1.9 (associated with DRw6,Dw9,DQw1) occurred in 46.4% and 22.2% of the patients with and without renal involvement, respectively (RR = 3.0, $P = 0.009$). The frequency of DR3 was slightly higher in the lupus nephritis patients compared with that in all groups except the black SLE patients, in whom the opposite was noted. The DR3 differences were significant only in the population of Asians, Polynesians, and Filipinos (see tables).

The allele frequency data from analysis of the 78 Caucasian SLE patients and 91 normal Caucasian controls (from Stanford) are shown in Table 2. Comparing patients with renal disease with those without renal disease identified several trends. For example, DQβ1.AZH and the DQβ subtypes of DRw6 were more frequent. However, only the increase in DQw1 (DQβ alleles associated with DR1, DR2, DRw6, or DRw10) and the decrease in DR4 in the lupus nephritis patients compared with those with SLE but without nephritis were statistically significant (RR = 3.0, $P = 0.04$ and RR = 0.28, $P = 0.02$, respectively).

Comparisons of lupus nephritis patients with healthy controls revealed significant increases in DR1 and DR2 (RR = 2.7 and 3.7, respectively). DQw1 was present in 84.1% of this group of patients (RR = 4.7, $P = 0.0003$), and 13.6% of them had the rare DR2-associated DQβ1.AZH allele, as compared with none of the 12 DR2 controls analyzed by SSO (RR = 30.9,

Table 3. Association of systemic lupus erythematosus (SLE) with DQβ polymorphic residues in Caucasian patients with and without nephritis*

		Association with SLE in Caucasians†		Polymorphic residues‡																					
				HVR_I					HVR_{II}							HVR_{III}									
DR	DQβ	Nephritis	No nephritis	26	27	28	29	30	52	53	54	55	56	57		66	67	68	69	70	71	72	73	74	75
1	1.1	Positive	Positive	G	V	T	R	H	P	Q	G	R	P	V		E	V	L	E	G	A	R	A	S	V
2	1.2	Positive	Positive	L	–	–	–	Y	–	–	–	–	–	D		–	–	–	–	–	T	–	–	E	L
	1.AZH	Positive	Positive	–	–	–	–	–	–	–	–	–	–	S		–	–	–	–	–	–	–	–	–	–
3	2	(Positive)	Neutral	L	–	S	–	S	L	L	–	L	–	A		D	I	–	–	R	K	–	–	A	–
w6	1.9	(Positive)	(Negative)	–	–	–	–	–	–	–	–	–	–	D		–	–	–	–	–	–	–	–	–	–
	1.18	(Negative)	(Negative)	L	–	–	–	–	–	–	–	–	–	D		–	–	–	–	–	T	–	–	E	L
	1.19	(Positive)	(Positive)	L	–	–	–	–	–	–	–	–	–	–		–	–	–	–	R	T	–	–	E	L
	1.SLE	(Positive)	(Positive)	L	–	–	–	Y	–	–	–	–	–	–		–	–	–	–	R	T	–	–	E	L
4	3.1	Negative	Neutral	Y	–	–	–	Y	–	L	–	P	–	D		–	–	–	–	R	T	–	–	E	L
	3.2	Negative	Neutral	L	–	–	–	Y	–	L	–	P	–	A		–	–	–	–	R	T	–	–	E	L
5	3.1	(Negative)	(Negative)	Y	–	–	–	Y	–	L	–	P	–	D		–	–	–	–	R	T	–	–	E	L
7	2	(Negative)	(Negative)	L	–	S	–	S	L	L	–	L	–	A		D	I	–	–	R	K	–	–	A	–
	3.1	(Negative)	(Negative)	Y	–	–	–	Y	–	L	–	L	–	D		–	–	–	–	R	T	–	–	E	L
w8	4(blank)	(Negative)	(Negative)	–	–	–	–	Y	–	L	–	–	L	D		D	I	–	–	E	D	–	–	–	–
9	3.3	(Negative)	(Negative)	L	–	–	–	Y	–	L	–	P	–	D		–	–	–	–	R	T	–	–	E	L

* See Table 1 and Patients and Methods for details of DR and DQβ determinations.

† See Table 2 for the relative risk values of the DQβ allele associations with the 2 subgroups. Parentheses indicate that the association is not statistically significant.

‡ Hyphens show residues that are identical to DQβ1.1 in hypervariable regions (HVR) I–III. Amino acid sequences are inferred from nucleotide sequences: DQβ1.1 (ref. 64), DQβ1.2 (refs. 23 and 32), DQβ1.AZH (refs. 23 and 32), DQβ2 (refs. 32 and 65), DQβ1.9 (refs. 26 and 27), DQβ1.18 (ref. 26), DQβ1.19 (refs. 32 and 66), DQβ1.SLE (refs. 31 and 32), DR4,DQβ3.1 (ref. 67), DR4,DQβ3.2 (ref. 68), DR5,DQβ3.1 (ref. 26), DR7,DQβ2 (refs. 32 and 69), DR7, DQβ3.1 (ref. 26), DR8,DQβ4(blank) (ref. 26), and DR9,DQβ3.3 (ref. 26).

$P < 0.005$). Direct inquiry of ethnic background and a review of patient and control surnames suggested that similar percentages of SLE patients and their controls were of Jewish descent. Because DwAZH is more prevalent in the Jewish population, we did this analysis to assure that the observed increase in the DQβ1.AZH allele in the lupus patients was independent of any ethnic skewing in our patient and control populations. Assuming the conservative estimate of the haplotype frequency for DQβ1.AZH as ~1% in the Caucasian population (12,48–50), the difference still yields a relative risk of 14.3 and a P value of 0.005.

Half of the white SLE patients with nephritis had the DQβ1.1, 1.AZH, or 1.9 alleles that share the first (amino acid positions 26–30) and third (amino acids 66–75) hypervariable regions in the DQβ first domain (see Tables 2 and 3). The first HVR (amino acids 26–30) shared by DQβ alleles 1.2 and the novel DQβ1.SLE (associated with DRw6,Dw?,DQw1) allele is seen in 39% of the lupus nephritis patients (RR = 2.7, $P = 0.008$ versus normal Caucasian controls).

Table 3 summarizes the association of SLE in Caucasians with specific DQβ polymorphic residues, subdivided into 3 HVRs. The DQβ1.2 and DQβ1.SLE alleles were not as nephritis-specific as the DQβ1.AZH allele in the Caucasian lupus patients.

In black SLE patients, however, the DQβ1.2 allele was highly associated with lupus nephritis, being present in 72.7% of these patients and in 22.2% of black SLE patients without renal involvement (RR = 9.3, $P = 0.03$) (Table 4). DQβ1.AZH was highly associated with lupus nephritis in black SLE patients (RR = 23.7, $P < 0.005$ versus normal black controls).

This association was even stronger in the Asian/Polynesian/Filipino SLE patients (Table 5). DR2 was observed in 78.6% of these lupus nephritis patients,

Table 5. Frequency (%) of HLA–DR, DQβ alleles in Asian, Polynesian, and Filipino SLE patients with nephritis and in Asian, Polynesian, and Filipino control subjects*

DR	DQβ	SLE with nephritis (n = 14)	Control subjects (n = 752)	RR	P
1	1.1	0	10		
2	1.2, 1.12, or 1.AZH	78.6	28	9.4	<0.005
	1.2	14.3	≤5.6		
	1.AZH	64.3	≤2.2	77.8	<0.005
3	2	28.6	3.5	11.2	<0.005
4	3.1	7.1	39.0	0.12	<0.05
5	3.1	14.3	21.0		
w6	1.9 or 1.18	14.3	19.0		
7	2	7.1	5.8		
w8	4(blank)	0	14.0		
9	3.3	7.1	21.0		
1, 2, or w6	DQw1	85.7	51.0	5.8	<0.025

* The control population for this group of patients was from the Ninth HLA Workshop, in 1984 (36), and the Third Asia–Oceania HLA Workshop, in 1986 (35). Only 2 patients in this ethnic group did not have nephritis, and their data are not included in this table. P values calculated by Woolf's formula (47). See Table 1 and Patients and Methods for details of DR and DQβ determinations, explanation of RR calculations, and explanations of abbreviations.

Table 4. Frequency (%) of HLA–DR, DQβ alleles in black SLE patients with and without nephritis and in black control subjects*

DR	DQβ	SLE with nephritis (n = 11)	SLE without nephritis (n = 9)	Control subjects (n = 323)	Lupus nephritis patients vs. patients without nephritis		Lupus nephritis patients vs. controls	
					RR	P	RR	P
1	1.1	18.2	0	9.6				
2†	1.2 or 1.AZH	81.8	22.2	28.5	15.8	0.01	11.3	<0.005
	1.2	72.7	22.2	26.0	9.3	0.03	7.6	<0.005
	1.AZH	18.2	0	1.0‡			23.7	<0.005
3	2	27.3	55.6	31.6				
4	3.1 or 3.2	18.2	11.1	9.6				
5	3.1	27.3	33.3	24.8				
w6	1.9, 1.18, 1.19 or 1.SLE	0	22.2	10.2			0.4	<0.005
7	2 or 3.1	9.1	11.1	18.6				
w8	4(blank)	0	22.2	10.8				
9	3.3	0	0	5.3				
1, 2, or w6	DQw1	81.8	44.4	55.2				

* The control population for this group of patients was from the Eighth HLA Workshop (North American black subjects) (see ref. 34). P values calculated by Woolf's formula (47), except for that of DRw6 in lupus nephritis patients versus controls, which was calculated using Haldane's modification of Woolf's formula for sets containing 0 (47). See Table 1 and Patients and Methods for details of DR and DQβ determinations, explanation of RR calculations, and explanations of abbreviations.

† The sum of the DR2-associated DQβ alleles is greater than the DR2 antigen frequency because several patients were heterozygous for DQβ1.2,1.AZH.

‡ Calculated assuming approximately 1% haplotype frequency in the normal black population (49).

compared with an expected frequency of 28% (35,36) (RR = 9.4, $P < 0.005$). Two-thirds (64.3%) of this group of lupus nephritis patients had the DQβ1.AZH allele, compared with an expected haplotype frequency for DQβ1.AZH of ≤2.2% (35,50) (RR = 77.8, $P < 0.005$). The increase in DQβ1.2 was not significant. DR3 occurred in 28.6% of these lupus nephritis patients, a significant increase, since this allele is present in only 3.5% of normal individuals in this ethnic group (RR = 11.2, $P < 0.005$). Among this group of SLE patients, there were only 2 who did not have nephritis; therefore, no meaningful comparisons of the 2 clinical subgroups can be made. DR4 was present in 1 of these 14 lupus nephritis patients (7.1%), and in this patient, the second allele was DQβ1.AZH. The background antigen frequency for DR4 in the normal population of this ethnic group is 39% (35,36).

HLA workshop controls are an imperfect control group because there may be differences between the patient and control populations that could potentially skew the interpretation of the results of such comparisons. We used these historical controls to compare our non-Caucasian patients because we were unable to obtain significant numbers of control subjects in each ethnic subset of lupus patients examined. The Asian, Polynesian, and Filipino patients were combined because the numbers of these patients were small and because HLA workshop data on these populations reveal similar antigen frequencies among individuals in this group. When the reported antigen frequencies differed, as in the case of DR2,Dw2 and DR2,DwAZH(Dw21) (35,49,50), we used the values at the higher end of the respective ranges to minimize the difference between patients and controls and thus avoid creating significance where none existed. Heterozygotes for DR2,w6 or for DR1,2 were increased among SLE patients in all ethnic groups, but the difference was not significant. Heterozygotes for DQw1,DQw2 were not significantly increased in any patient group.

Complement genes and their products. C4A deficiency, as measured by protein assay and/or by RFLP, occurred in one-third of the patients; 39.7% of the patients with renal disease and 28.6% of the patients without renal disease were C4A null. This difference was not significant. Overall, 35 of 99 SLE patients (35.4%) were C4A deficient. C4A null occurred in 24 of 62 Caucasian SLE patients (38.7%), compared with a frequency of 31.8% in the Caucasian controls (n = 960). This difference is not statistically significant. No significant ethnic differences in the associations with C4A were noted.

Hind III digests of genomic DNA from SLE patients show normal C4A and C4B patterns (i.e., the presence of 15-kb and 25-kb bands) after probing with a C4 cDNA probe. A C4A gene deletion is detected by the appearance of a new 8-kb band. Table 6 summarizes the HLA–DR and DQβ associations with the 2 forms of C4A deficiency observed. Only the patients in whom unequivocal assignment of the C4A allele (by immunofixation electrophoresis) could be made were included. Approximately two-thirds of the patients had a DR3-associated C4A gene deletion, which accounts for the absence of the C4A protein. The remaining one-third of the C4A-null patients had a normal RFLP pattern, which suggests a different mechanism of C4A deficiency. It is conceivable that studies of families could classify more patients into the C4A-null subset of normal RFLP and absent C4A gene product. The C4A-null subset with normal RFLP occurred primarily in patients who were DR2 positive and almost exclusively in those with the DQw1 allele.

C2 deficiency is associated with a normal RFLP pattern (data not shown and ref. 52). Without family studies, the C2 protein assay cannot unequivocally separate acquired C2 deficiency (secondary to the

Table 6. HLA class II alleles in SLE patients without C4A protein*

Absent C4A protein (n = 31)	n	No. (%) positive for HLA class II allele
Abnormal RFLP, C4A gene deletion	20	12 (60) DR3
Normal C4A RFLP	11	10 (91) DQw1 (associated with DR1, 2, or w6)
		7 (63.6) DR2
		3 DQβ1.AZH
		4 DQβ1.2
		2 (18.2) DRw6, DQβ1.19
		1 (9.1) DR1, DQβ1.1
		1 (9.1) DR3, 7, DQβ2

* See Results for details. SLE = systemic lupus erythematosus; RFLP = restriction fragment length polymorphism.

activation and consumption of complement, as is seen with active SLE) from the inherited form; therefore, the overall frequency of C2 deficiency (15%) is probably an overestimation of the true frequency of inherited C2 deficiency. (In the normal Caucasian population, C2 deficiency occurs in 1% [53].) Sixty-four percent of our C2-deficient patients were DR2 positive.

Genotype and phenotype analysis of factor B and C4B null in our lupus patient population yielded values that were similar to those in the control population (data not shown).

DISCUSSION

The clinical subset of lupus nephritis revealed a more genetically homogeneous patient group than did the SLE population as a whole. Most of the significant differences held across ethnic groups. These findings underscore the importance of clinical subsetting in heterogeneous autoimmune disorders such as SLE when searching for genetic predisposition.

The increase in the normally rare DR2-associated DQβ1.AZH allele was significant in the lupus nephritis population. Although significantly increased in Caucasian patients (RR = 14) and in black patients (RR = 24), DQβ1.AZH was present in <20% of those who had nephritis. In contrast, DQβ1.AZH was the predominant SLE nephritis allele in the Asian, Polynesian, and Filipino patients who had lupus nephritis (64% versus ≤2.2%; RR = 78) as compared with their ethnically matched controls.

The association between DQβ1.AZH and lupus nephritis is much stronger than the previously reported associations of lupus with any single MHC allele. The previously reported relative risk values tend to be ≤3 for these alleles when considered individually (6–9). Higher relative risks have been reported only in settings of multiple MHC alleles such as DR2 and C4A null (RR = 25) (9) and the restricted dermatologic subset of SLE, subcutaneous lupus erythematosus with HLA–DR3 (RR = 12) (54). In our study, the relative risk values of 14, 24, and 78 in the respective subgroups of the Caucasian, the black, and the Asian, Polynesian, and Filipino patients with nephritis suggest the importance of this allele in the nephritis component of SLE.

Comparison of the polymorphic first domain sequence of this DQβ allele with other DQβ alleles that are overrepresented in these patients (DQβ1.1 and 1.9) suggests that the first HVR (amino acids 26–30, residues GVTRH) and the third HVR (amino acids 66–75, residues EVLEGARASV) may be important in predisposition to lupus nephritis (see Table 3). These residues are shared by the DQβ alleles 1.1, 1.AZH, and 1.9. All 3 alleles were increased in Caucasian lupus nephritis patients; however, only DQβ1.1 and DQβ1.AZH were independently associated with nephritis. DR2,DQβ1.AZH and DRw6,DQβ1.9 are both quite rare in the normal Caucasian population (normal haplotype frequency ~1% and ~4%, respectively [12,48–50]) and have been found to be associated with other autoimmune disorders: 1.AZH with insulin-dependent diabetes mellitus (20,26) and 1.9 with pemphigus vulgaris (27,28). Residues 28 and 30 are at the bottom of the antigen-binding cleft, pointing upwards into the site, according to the hypothesized 3-dimensional model of the class II molecule (55); this implicates involvement of the first HVR in antigen interaction. Polymorphic residues 70 and 71 point upwards and, presumably, interact with a specific T cell receptor. Sharing of these polymorphic residues may explain the high association of the DQβ1.AZH allele in the lupus nephritis population and the combination of DQβ alleles 1.1, 1.AZH, and 1.9 in 50% of the Caucasian, 27% of the black, and 71% of the Asian, Polynesian, and Filipino lupus nephritis patients.

Other investigators utilizing RFLP analysis to study SLE patients have not found a significant increase in the DQβ1.AZH allele (56,57). Possibly, they analyzed a different genetic subset of SLE. It is more likely, however, that the difference is due to their use of RFLP analysis alone. The DQβ1.AZH RFLP may be masked if the second allele is DQβ1.2 or 1.18 (associated with DRw6,Dw18,DQw1) or 1.19 (associated with DRw6,Dw19,DQw1); thus, the frequency of the DQβ1.AZH allele would be underestimated, unless SSO analysis is employed. Five patients in our group were heterozygous for DQβ1.AZH, 1.2 or 1.18 or 1.19. The DQβ RFLP from these patients suggested the common DR2-associated DQβ1.2 allele. Only after SSO analysis with a 1.AZH sequence-specific oligonucleotide probe could the 1.AZH allele frequency be appreciated in these heterozygous patients.

DR2 was predominant in the black SLE patients (82%; RR = 16) and in the Asian, Polynesian, and Filipino SLE patients (79%; RR = 9) and was significantly increased in the Caucasian SLE patients (48%; RR = 4). The predominant Caucasian lupus nephritis "supratype" was DQw1 (84%; RR = 5) (associated with DR1,DQβ1.1, DR2,DQβ1.2, or

Table 7. Summary of class II and class III major histocompatibility complex associations with lupus nephritis in a group of Caucasians, a group of blacks, and a group of Asian, Polynesian, and Filipino patients versus ethnically matched normal control groups*

	RR for lupus nephritis vs. ethnically matched controls			RR for lupus nephritis vs. patients without nephritis (all groups)
	Caucasians	Blacks	Asians, Polynesians, Filipinos	
Class II allele				
DR2,DQβ 1.2, 1.12, or 1.AZH	3.7	11.3	9.4	2.6
DR2,DQβ1.2	2.7	7.6	(2.8)	(1.3)
DR2,DQβ1.AZH	14.2	23.7	77.8	5.6
DR4,DQβ 3.1 or 3.2	0.25	0.4	0.12	0.34
DQw1,DQβ 1.1, 1.2, 1.12, 1.AZH, 1.9, 1.18, 1.19, or 1.SLE	4.7	(3.7)	5.8	5.3
Class III allele				
C4A null				
C4A gene deletion				
Associated with HLA-DR3				
Abnormal regulation of C4A expression (?)				
Associated with HLA-DQw1				

* C4A was characterized by protein electrophoresis and restriction fragment length polymorphism analysis. See Table 1 and Patients and Methods for details of DR and DQβ determinations, explanations of statistical calculations, and explanations of abbreviations. RR values in parentheses are not statistically significant; all other RR values are significant at $P \leq 0.02$.

DQβ1.AZH or DQβ1.12, and DRw6,DQβ1.9, 1.18, 1.19, or 1.SLE) (see Table 7).

Although the use of historical controls for the non-Caucasian populations is not ideal, we included these data because of the unexpected, very high incidence of the DQβ1.AZH allele, especially in the group of Asian, Polynesian, and Filipino patients with lupus nephritis, which served to complement the immunogenetic data in the well-controlled Caucasian population with lupus nephritis. Further analyses of the Asian, Polynesian, and Filipino lupus nephritis patients in comparison with ethnically matched California controls will need to be done to determine whether the observed association remains highly significant.

Interestingly, the presence of HLA-DR4 was significantly diminished in the lupus nephritis patients of all ethnic groups in comparison with the SLE patients without renal disease. This decrease in DR4 has been observed previously in Japanese patients with SLE (58). In our lupus nephritis patients, the decrease in DR4 was not selective of a particular DR4-associated DQβ allele. The distribution of DQβ3.2 to DQβ3.1 was the same as in the normal population. It is conceivable, however, that the lupus nephritis "protective" effect is related to the third HVR of the DRβ chain, as in rheumatoid arthritis (59) and pemphigus vulgaris (28), or, possibly, to another linked gene. These hypotheses remain to be tested.

Protein and RFLP analyses confirmed the occurrence of the previously reported associated deficiencies of C4A, C4B, and C2. These deficiencies were not associated with major differences within the various patient groups or in the patients versus the controls. However, it was an analysis of the discrepancies between the C4A protein and RFLP data that revealed several interesting associations. Two-thirds of the C4A-null patients had RFLP results consistent with the reported C4A gene deletion (29) together with undetectable C4A protein. Most of these patients were DR3 positive, as would be anticipated from linkage disequilibrium and the "autoimmune" extended haplotype A1,B8,DR3,SC01 (60). The remaining one-third of the patients who would traditionally be characterized as C4A null because of the absence of C4A protein had normal RFLP results, which indicates that this gene is not deleted in these 11 lupus patients. Most of these patients were also DR2 posi-

tive, and nearly all were positive for DQw1. Although this result does not exclude the possibilities of a minor sequence change in the coding or in the regulatory portion of the C4A gene preventing proper transcription and/or translation of the C4A gene, it is intriguing to speculate that a DR2,DQw1-linked gene with broader regulatory effects is a susceptibility allele for a subset of SLE patients.

Supporting experimental evidence suggests that this gene may be tumor necrosis factor α (TNFα). Activated peripheral blood lymphocytes from DR2-positive and especially DQw1-positive lupus nephritis patients have lower TNFα inductibility than do those from DR3-positive or DR4-positive patients (61). New Zealand white (NZW) mice and (New Zealand black × NZW)F_1 ([NZB × NZW]F_1) mice have previously been demonstrated to have low TNFα inductibility, and intraperitoneal injections of TNFα into (NZB × NZW)F_1 mice delayed the onset of lupus nephritis and prolonged survival in this strain of lupus-susceptible mice (62). These data from the study of mice and our data from the study of humans suggest that this MHC-linked cytokine may have a role in lupus nephritis susceptibility. Although TNFα is known to up-regulate the expression of components of the alternative pathway of complement (C3 and factors B and H), its effect on several of the proteins of the classical pathway of complement (C1r, C1s, C1 inhibitor, and C2) is minimal (63). A specific effect of TNFα on C4A is not known.

Perhaps SLE is triggered by the interaction of a particular foreign antigen with a unique subset of DQβ alleles (DQβ1.AZH, 1.1, 1.9, or 1.2 and 1.SLE) and T cell receptor(s). The disease may then be perpetuated by an MHC-linked regulatory abnormality, such as a TNFα variant effecting abnormal expression of complement or altered T cell subset differentiation. Family studies, analysis of other MHC loci, and class II and III MHC regulation experiments should further elucidate the role of these genes in SLE susceptibility.

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