Association of Amino Acid Sequences in the HLA-DQB1 First Domain with the Antitopoisomerase I Autoantibody Response in Scleroderma (Progressive Systemic Sclerosis)

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Abstract

Previous studies in Caucasians with progressive systemic sclerosis (PSS) have suggested associations of antitopoisomerase I (antitopo I) autoantibodies with either serologically defined HLA-DR2 or DR5. To better define class II HLA associations with the antitopo I response, 161 PSS patients (132 Caucasians and 29 American blacks) were studied for antitopo I autoantibodies by immunodiffusion and immunoblotting, and their HLA-DRB1, DRB3, DQA1, and DQB1 alleles were determined by restriction fragment length polymorphic analysis and DNA oligotyping. Among Caucasians with antitopo I, HLA-DR5(DRB1*1101–*1104), DRB3*0202 and DQw3 (DQw7,8,9) were significantly increased in frequency. In American blacks, however, only HLA-DQB1*0301 (DQw7) was significantly increased. The presence of HLA-DQB1*0301 (DQw7) and other HLA-DQB1 alleles bearing the uncharged polar amino acid residue tyrosine at position 30 of the outermost domain was found in all antitopo I–positive Caucasian PSS patients compared with 66% of antitopo I–negative PSS patients (pc = 0.007) and 70% of normal controls (pc = 0.008), as well as all antitopo I–positive black patients. The association with HLA-DQB1 was independent of HLA-DR5(DRB1*1101–*1104) or any other HLA-DRB1, DRB3, or DQA1 alleles. Alternative or additional candidate epitopes for this autoimmune response include alanine at position 38 and threonine at position 77 of these same DQB1 alleles. These data suggest that genetic predisposition to the antitopo I response in PSS is associated most closely with the HLA-DQB1 locus. (*J. Clin. Invest.* 1992. 90:973–980.) Key words: immunogenetics • autoimmunity • nucleoprotein • genotype • autoantigen

Introduction

The cause of progressive systemic sclerosis (PSS),[1] or scleroderma, is as yet unknown, although both genetic and environmental factors have been implicated (1–7). A number of studies have reported associations of HLA antigens with PSS, including *MHC class I specificities:* HLA-A9 (8, 9), B8 (10, 11), and B35 (12, 13); *MHC class II specificities:* HLA-DR1 (13–16), DR3 (11, 17), and DR5 (16, 18–20); and *MHC class III specificities:* C4 null alleles (12, 19). These associations have varied among different centers, with some groups unable to show any HLA associations with PSS (21–25).

PSS is a disease in which several highly disease-specific autoantibodies have been defined, which in turn are associated with certain clinical features (26, 27). These include anticentromere autoantibodies (ACA), occurring predominantly in the CREST variant or mild scleroderma (26–33); antinucleolar autoantibodies, including anti-PM-Scl, associated with the presence of myositis in PSS patients (34); and antitopoisomerase I (antitopo I), otherwise known as anti-Scl-70, which correlates with widespread skin involvement and systemic disease (26, 27, 35). Several recent reports have correlated autoantibody subsets more strongly with certain MHC-class II alleles, including ACA with HLA-DR1 (27, 36), DR4 (36), DR5(DRw11) (20), and DRw8 (36); anti-PM-Scl with HLA-DR3 (36); and antitopo I with HLA-DR2 (25) and DR5(DRw11) (27, 36). Few studies, however, have examined the expanded polymorphism at the molecular level now recognized for the adjacent HLA-DQ loci, although one study reported an increased frequency of HLA-DQw7 in patients with ACA (20).

Using restriction fragment length polymorphisms (RFLPs) and oligonucleotide typing of class II MHC alleles, we have reported recently that HLA-DQB1 alleles possessing a polar amino acid (either tyrosine or glycine) at position 26 of the outermost domain constitute the strongest association with the ACA autoimmune response in PSS patients (37). In the present study, we used the same techniques in a large number of PSS patients to compare HLA-DRB1, DRB3, DQA1, and DQB1 alleles in antitopo I–positive and –negative PSS patients and controls to show that the antitopo I response also is correlated most closely with specific HLA-DQB1 chain alleles possessing other shared amino acid sequences.

Methods

Patients and controls. As an ongoing study, all patients with PSS seen by members of the Divisions of Rheumatology at the University of Texas Health Science Center at Houston (J.D.R., F.C.A.), Ottawa General Hospital, the University of Ottawa (R.G.), and the University of Miami School of Medicine (R.M., R.D.A.) are studied for a variety of autoantibodies and undergo HLA-DR, DQ, and DP typing by RFLP and/or oligotyping methods in the University of Texas Laboratories after informed consent is obtained. To date 161 patients with PSS have been evaluated, including 74 from Houston, 34 from Ottawa, and 44

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1. *Abbreviations used in this paper:* ACA, anticentromere autoantibodies; antitopo I, antitopoisomerase I; OR, odds ratio; PC, corrected *P* value; PCR, polymerase chain reaction; PSS, progressive systemic sclerosis; RFLP, restriction fragment length polymorphism.

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from Miami, as well as 9 from Georgia who were part of an epidemiological study reported previously (38). 132 were Caucasian and 29 were American black. In addition, three native Americans (Choctaws) with PSS were investigated. All patients had either PSS by the preliminary criteria formulated by the American College of Rheumatology (39), or the CREST syndrome if at least three out of the five CREST features (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) were observed during the course of the disease.

Normal controls included 200 local Houston Caucasians of predominantly northwestern European ancestry and 88 local American black volunteers who were blood donors or medical school personnel and whose HLA-DR and DQ alleles were defined similarly by RFLP and oligotyping analyses.

Autoantibody determinations. ACA and antinuclear antibodies were determined by indirect immunofluorescence on Hep-2 cells, with positivity defined at serum dilutions 1:80 or greater. ACA were detected by the typical immunofluorescent pattern seen on interphase and metaphase nuclei of Hep-2 cell substrate. Antitopo I antibodies were determined by both passive immunodiffusion using calf thymus extract as substrate and by immunoblotting of affinity-purified topoisomerase I from HeLa cell nuclei (E.D.) (40) at the Baylor College of Medicine.

Genetic analysis of HLA-DRB1, DRB3, DQA1, and DQB1 alleles. Genomic DNA was extracted from peripheral blood leukocytes (41). "Broad" HLA-DR specificities (DR1, DR2, DR3/6, DR4, DR5(DRw11), DR5(DRw12), DR7, DRw8, DR9, and DRw10) were determined either by RFLP analysis of *Taq* I, *Bam* HI and *Eco* RI digested genomic DNA described previously (42) or by polymerase chain reaction (PCR) amplification of 0.5 μg of genomic DNA using the primers DRBAMP-A (5'-CCCCACAGCACGTTTCTTG-3') and DRBAMP-B (5'-CCGCTGCACTGTGAAGCTCT-3'). The PCR was carried out for 30 cycles under the following parameters: denaturation 1 min at 96°C, annealing 1 min at 55°C, extension 2 min at 72°C. Hybridization and washing conditions were as described previously (43).

For the RFLP analyses, HLA-DR and DQ specificities were assigned to specific RFLP bands at the 10th International Histocompatibility Testing Workshop (44), as well as in our own (45, 46) and others' studies (47–50). Specific HLA-DQA1 and DQB1 chain alleles in each individual were assigned by comparison with reported DQA1 and DQB1 RFLPs for HLA-D haplotypes as determined by the World Health Organization Nomenclature Committee for Factors of the HLA System (44, 51).

For HLA-DR5 subtyping, patients and controls positive for HLA-DR3(DRw17), DR5(DRw11), DR5(DRw12), DRw6(DRw13,14), and DRw8 by RFLP or oligotyping underwent group-specific PCR amplification of DRB1 alleles using the primers DRBAMP-3 (5'-CACGTTTCTTGGAGTACTCTAC-3') and DRBAMP-B under conditions outlined for DRBAMP-A and B, except the annealing step for the PCR was at 60°C for 30 s. HLA-DR1 and DR4 subtypes were determined by group-specific amplification of HLA-DRB1 alleles using the primers DRBAMP-1 (5'-TTCTTGTGGCAGCTTAAGTT-3') and DRBAMP-4(5'-GTTTCTTGGAGCAGGTTAAAC-3') along with DRBAMP-B under the same PCR conditions (37). HLA-DRB3(DRw52) alleles were determined by PCR amplification with the primers DRBAMP-52 (5'-CCCAGCACGTTTCTTGGAGCT-3') and DRBAMP-B. HLA-DR2-DRB1 alleles were determined as has been described elsewhere (43).

18-mer oligonucleotide probes, supplied by the 11th International Histocompatibility Testing Workshop, were used to detect polymorphic sequences around positions 28, 37, 57, 70, and 86 of the DRB1 first domain to distinguish HLA-DR1(DRB1*0101–*0103), DR4(DRB1*0401–*0411), and DRB3(DRw52) alleles, as well as DRB1*1101, *1102, *1103, and *1104 (as well as subtypes of HLA-DR3, DRw6, and DRw8) similar to those described elsewhere (52, 53).

For HLA-DQA1 typing, genomic DNA was amplified with the primer pair DQAAMP-A (5'-ATGGTGTAAACTTGTACCAGT-3') and DQAAMP-B (5'-TTGGTAGCAGCGGTAGAGTTG-3') under conditions outlined for DRB, except the annealing step for the PCR was for 1 min at 55°C. Oligonucleotide probes corresponding to the polymorphisms at positions 25, 34, 55, and 69 were used for the hybridizations as supplied by the 11th International Histocompatibility Testing Workshop.

For HLA-DQB1 typing, the primers used included DQBAMP-A (5'-CATGTGCTACTTCACCAACGG-3') and DQBAMP-B (5'-CTGGTAGTTGTGTCTGCACAC-3') under the same PCR conditions as the DQA1 primers. Oligonucleotide probes corresponding to the polymorphic codons 26, 37, 45, 49, 57, and 70 were used for dot blot hybridization.

Statistical analyses. Comparisons of HLA specificities and allelic frequencies in antitopo I–positive versus antitopo I–negative PSS patients and normal race-matched controls were performed using the chi square analysis of 2×2 tables and Yates' correction with the EPI-INFO statistical program. When one of the groups being analyzed contained five or less members, a two-tailed Fisher's exact test was used. Significant P values were corrected (pc) by multiplying the number of comparisons, i.e., for DR specificities $\times$ 12, DQ specificities $\times$ 8, DRB1 alleles $\times$ 40, DQA1 alleles $\times$ 7, DQB1 alleles $\times$ 12, and DPB1 alleles $\times$ 19. Where an association with a particular specificity had been reported previously, the P value was not corrected. Odds ratios (OR) were also determined from the same 2×2 tables.

Results

Scleroderma-related autoantibody frequencies. Of the 132 Caucasians with PSS examined, antitopo I autoantibodies were found in 21 (16%). No significant differences occurred in the frequencies of antitopo I in Caucasian patients from Houston, Ottawa, and Miami. On the other hand, antitopo I antibodies were found in 38% of the 29 American blacks with PSS, a frequency that was significantly increased in blacks compared with Caucasians, as we have reported previously (54). All but three of the patients with antitopo I were positive by both immunodiffusion and immunoblotting, with three being positive by immunoblotting only. ACAs were found in only one black PSS patient, a frequency significantly less than the 36% seen in Caucasians (54).

Clinical differences between autoantibody subgroups. Medical records were retrospectively reviewed in 126 (95%) of the 132 Caucasians with PSS. Differences in clinical features between the autoantibody subsets of PSS are shown in Table I. Proximal/diffuse scleroderma (skin involvement proximal to the metacarpophalangeal joints) and interstitial pulmonary disease were significantly less frequent in Caucasians with ACA than in PSS patients lacking this autoantibody (the latter reflecting what has been observed elsewhere [32]).

Among 28 American blacks in whom medical records were reviewed, diffuse or proximal scleroderma and interstitial pulmonary fibrosis were slightly more frequent in those with antitopo I than in those without, although the differences were not significant, perhaps because of the small numbers examined (clinical data were not available on the one black with ACAs).

HLA class II frequencies in Caucasians. All but five Caucasian PSS patients and 10 controls had sufficient genomic DNA available for oligotyping. In the few remaining patients and controls, HLA-DRB1, DQA1, and DQB1 alleles were determined by RFLP analysis, with alleles being assigned by linkage disequilibrium with characteristic RFLP patterns (44).

Table I. Clinical Features in Autoantibody Subsets of Progressive Systemic Sclerosis

	Antitopo I*	ACA*	Other PSS
Caucasians	(n = 21)	(n = 45)	(n = 62)
Proximal/diffuse scleroderma‡*	18 (86%)	18 (40%)‡	47 (77%)
Pulmonary fibrosis	12 (57%)	1 (2%)‡	23 (37%)
Renal involvement§	1 (5%)‖	1 (2%)‖	7 (11%)
Myositis	0 (0%)	0 (0%)	9 (15%)
American blacks	(n = 11)	(n = 0)	(n = 17)
Proximal/diffuse scleroderma*	11 (100%)	—	14 (82%)
Pulmonary fibrosis	7 (64%)	—	6 (35%)
Renal involvement§	0 (0%)	—	3 (18%)
Myositis	0 (0%)	—	3 (18%)

* Defined as skin involvement proximal to metacarpophalangeal joints. ‡ $P < 0.001$ compared with either antitopo I–positive or AACA-positive patients. § Defined as rapidly progressive renal insufficiency and hypertension. ‖ Including one patient with both ACA and antitopo I, who had sclerodermatous renal involvement.

Table II. HLA-DRB1 Frequencies in Autoantibody Subsets of Progressive Systemic Sclerosis in Caucasians

HLA-DRB1	Antitopo I‡ (n = 21)	ACA‡ (n = 47)	Other PSS (n = 65)	Controls (n = 200)
*0101(DR1)§	1 (5%)	14 (30%)	12 (18%)	35 (18%)
*0102(DR1)	1 (5%)	5 (11%)	4 (6%)	9 (5%)
*0103(DR1)	1 (5%)	2 (4%)	2 (3%)	12 (6%)
*1501(DR2)	7 (33%)	6 (13%)	11 (17%)	51 (26%)
*1502(DR2)	0 (0%)	0 (0%)	1 (2%)	5 (3%)
*1601(DR2)	1 (5%)	3 (6%)	0 (0%)	3 (2%)
*1602(DR2)	0 (0%)	0 (0%)	1 (2%)	0 (0%)
*0301(DR3)	3 (14%)	6 (13%)	25 (38%)	40 (20%)
*0401(DR4)	4 (19%)	8 (17%)	7 (11%)	29 (15%)
*0402(DR4)	1 (5%)	1 (2%)	2 (3%)	5 (3%)
*0403(DR4)	0 (0%)	4 (9%)	2 (3%)	6 (3%)
*0404(DR4)	2 (10%)	1 (2%)	4 (6%)	7 (4%)
*0405(DR4)	2 (10%)	0 (0%)	0 (0%)	1 (1%)
*0407(DR4)	0 (0%)	4 (9%)	0 (0%)	5 (3%)
*0408(DR4)	0 (0%)	0 (0%)	1 (2%)	2 (1%)
*1101–*1104(DR5)	10 (48%)‖¶	14 (30%)	9 (14%)	21 (11%)
*1101(DR5)	6 (29%)	7 (15%)	3 (5%)	13 (7%)
*1102(DR5)	0 (0%)	0 (0%)	2 (3%)	2 (1%)
*1103(DR5)	1 (5%)	1 (2%)	0 (0%)	2 (1%)
*1104(DR5)	3 (14%)	7 (15%)	5 (8%)	5 (3%)
*1201(DR5)	0 (0%)	0 (0%)	1 (2%)	1 (1%)
*1301(DRw6)	0 (0%)	3 (6%)	2 (3%)	27 (14%)
*1302(DRw6)	1 (5%)	3 (6%)	7 (11%)	21 (11%)
*1303–*1305(DRw6)	0 (0%)	0 (0%)	1 (2%)	2 (1%)
*1401(*DRw6)	0 (0%)	3 (6%)	4 (6%)	9 (5%)
*1402(DRw6)	1 (5%)	2 (4%)	2 (3%)	4 (2%)
*0701(DR7)	5 (24%)	3 (6%)	13 (20%)	41 (21%)
*0801(DRw8)	1 (5%)	5 (11%)	4 (6%)	13 (7%)
*0802(DRw8)	0 (0%)	0 (0%)	1 (2%)	0 (0%)
*0803(DRw8)	0 (0%)	2 (4%)	0 (0%)	1 (1%)
*0804(DRw8)	0 (0%)	0 (0%)	4 (6%)	4 (2%)
*0901(DR9)	0 (0%)	0 (0%)	1 (2%)	4 (2%)

‡ One patient had both ACA and antitopo I, associated with HLA-DR3(DRw17), DQw2.1, and HLA-DRw8, DQw4 specificities and is included in both columns. § HLA-DR specificities composed of the respective HLA-DRB1 alleles are shown in parentheses. HLA-DRB1*0406, *0409–*0411(DR4), DRB1*1403–*1405(DRw6), and DRB1*1001(DRw10) were found in none of the patients and are thus not shown. ‖ $P = 0.002$, OR = 6 compared with other PSS patients. ¶ $P = 0.00001$, OR = 8 compared with other PSS controls.

HLA-DR and DQ specificities and alleles did not differ significantly between those with or without proximal/diffuse scleroderma (data not shown). HLA-DQw5 was marginally decreased in those with interstitial pulmonary fibrosis (24 vs. 48% in those without, $P = 0.02$ uncorrected). This probably reflected the fact that HLA-DQw5 was weakly associated in this group with ACAs (55 vs. 31% in ACA-negative PSS patients, $P = 0.01$ uncorrected, and vs. 34% in controls, $P = 0.008$ uncorrected), which were nearly absent in those with interstitial pulmonary fibrosis. No significant differences in HLA-DR or DQ frequencies were found in those with renal or gastrointestinal involvement or pulmonary hypertension, although the small numbers of patients in these groups (< 10 in each) prevented meaningful statistical analysis.

The frequencies of HLA-DR2(DRB1*1501–*1602), DR4(DRB1*0401–*0405) and DR5(DRw11)(DRB1*1101–*1104) were increased in Caucasian PSS patients with antitopo I compared with either PSS patients lacking antitopo I or ACAs (other PSS patients in Tables II and III) or controls, although only the broad specificity HLA-DR5(DRB1*1101–*1104) achieved statistical significance. It should be noted that ACA-positive PSS patients were considered separately because of the association of this autoantibody response also with HLA-DQB1*0301(DQw7) (37) (Table III).

Oligotyping for subtypes of HLA-DR5(DRw11) showed equivalent frequencies of DRB1*1101–*1104 in antitopo I positives and race-matched controls (Table II). Similarly, HLA-DR2(DRB1*1501–1602) and DR4(DRB1*0401–*0411) subtypes did not differ significantly between antitopo I–positive patients and racially matched controls.

HLA-DQB1*0301(DQw7) was increased in frequency in Caucasians with antitopo I compared with other PSS patients ($P = 0.02$), as well as to Caucasian controls ($P = 0.009$), although these comparisons did not withstand correction for the number of variables examined ($n = 8$) (Table III). HLA-DQB1*0302(DQw8) and DQB1*0303(DQw9) were also increased, but again not significantly. Of the nine HLA-DQB1*0301(DQw7)-negative Caucasian PSS patients with antitopo I, six (67%) had either HLA-DQw8 or DQw9. Of the three remaining antitopo I–positive patients, one was homozygous for HLA-DQw6, one had DQw5 and DQw6, and the third had HLA-DQw2.1 and DQw4. The latter individual was the only PSS patient found to have both antitopo I and ACA autoantibodies. HLA-DQB1*0301(DQw7), *0302(DQw8) and *0303(DQw9) are the DQβ chains that constitute the broad specificity DQw3, and this specificity was significantly increased in frequency compared with either other PSS patients and controls (Table III). If the allelic frequency of HLA-DQw3 was considered, statistical significance was maintained, even

Table III. HLA-DQA1 and DQB1 Frequencies in Autoantibody Subsets of Progressive Systemic Sclerosis in Caucasians

	Antitopo I‡ (*n* = 21)	ACA‡ (*n* = 47)	Other PSS (*n* = 65)	Controls (*n* = 200)
HLA-DQA1				
*0101	3 (14%)§	25 (53%)	22 (34%)	64 (32%)
*0102	8 (38%)	12 (26%)	19 (29%)	74 (37%)
*0103	1 (5%)	3 (6%)	3 (5%)	35 (17%)
*0201	2 (10%)	3 (6%)	14 (22%)	40 (20%)
*03	9 (43%)	16 (34%)	19 (29%)	61 (32%)
*0401	1 (5%)	5 (11%)	5 (8%)	12 (6%)
*0501	13 (62%)	18 (38%)	35 (54%)	71 (36%)
HLA-DQB1				
*0501–*0503(DQw5/DQw1)*	4 (19%)	26 (55%)	23 (35%)	67 (34%)
*0501	3 (14%)	21 (45%)	18 (28%)	57 (29%)
*0502	1 (5%)	2 (4%)	0 (0%)	3 (2%)
*0503	0 (0%)	4 (9%)	4 (6%)	8 (4%)
*0601–*0604(DQw6/DQw1)*	8 (38%)	12 (26%)	22 (34%)	102 (51%)
*0601	0 (0%)	2 (4%)	1 (2%)	5 (3%)
*0602	7 (33%)	6 (13%)	11 (17%)	52 (26%)
*0603	0 (0%)	2 (4%)	2 (3%)	32 (16%)
*0604	1 (5%)	3 (6%)	8 (12%)	23 (12%)
0201(DQw2)	5 (24%)	7 (15%)	33 (51%)	72 (36%)
*0301–*0303(DQw3)*	18 (86%)‖¶	29 (62%)	31 (48%)	92 (46%)
0301(DQw7/DQw3)	12 (57%)	25 (53%)**‡‡	17 (26%)	54 (27%)
0302(DQw8/DQw3)	7 (33%)	7 (15%)	12 (18%)	34 (17%)
0303(DQw9/DQw3)	3 (14%)	2 (4%)	5 (8%)	10 (5%)
0402(DQw4)	1 (5%)	5 (9%)	5 (8%)	13 (7%)

* Numbers in parentheses refer to specific/broad HLA-DQ specificities composed of the DQB1 allele shown. No patients had HLA-DQA1*0601 (not shown). ‡ One patient had both ACA and antitopo I, associated with HLA-DRB1*0301, DQA1*0501, DQB1*0201, and HLA-DRB1*0801, DQA1*0401, DQB1*0402 and is included in both columns. § pc = 0.04 compared with ACA positive patients. ‖ pc = 0.04, OR = 6.6 compared with other PSS patients. ¶ pc = 0.016, OR = 6.7 compared with controls. ** *P* = 0.009, OR = 3 compared with other PSS patients. ‡‡ *P* = 0.001, OR = 3 compared with controls.

after correcting for the eight HLA-DQ specificities examined (57% in antitopo I–positive vs. 29% in other PSS patients (pc = 0.01) and 26% in controls [pc = 0.0004, OR = 3.8]).

HLA-DQA1 alleles were of similar frequency in antitopo I–positive PSS patients, other PSS patients, and controls (Table III), as were HLA-DPB1 allele frequencies (55). HLA-DRB3*0202(DRw52b) was also slightly increased in frequency in antitopo I–positive (52%) compared with antitopo I–negative PSS patients (32%, *P* = NS) and controls (21%, pc = 0.012) (data not shown). No differences in the frequencies of HLA-DR5(DRw11), DQB1*0301(DQw7), and DQw3 were seen between patients from Texas, Ottawa, or Florida (data not shown).

HLA-class II frequencies in American blacks. All American black PSS patients and controls were typed for HLA class II alleles by oligotyping. Neither the extent of skin involvement nor the presence of interstitial pulmonary fibrosis correlated with HLA-DR or DQ specificities among American black PSS patients with antitopo I. On the other hand, the broad specificities HLA-DR4 (DRB1*0401, *0403, *0407) and DR5(DRB*1101–*1104) were both increased in frequency in black antitopo I–positive patients compared with other PSS patients or black controls, although not significantly so, perhaps because of the small number of black PSS patients (Table IV). HLA-DR5(DRw11) and DR4 subtype frequencies did not significantly differ in the antitopo I–positive patients from that in the other PSS patients or controls. HLA-DQB1*0301(DQw7), however, was associated with the antitopo I response compared with both antitopo I–negative PSS patients and controls (Table V). Of the two HLA-DQB1*0301(DQw7)–negative American black PSS patients, one had HLA-DQB1*0501 (DQw5) and DQB1*0602(DQw6) and the other had HLA-DQB1*0201(DQw2) and DQB1*0402(DQw4). HLA-DQB1*0602(DQw6) was actually decreased in frequency in antitopo I–positive vs.–negative PSS patients, although not significantly.

Localization of susceptibility to antitopo I to specific HLA-DQB1 sequences. As shown above, most of the Caucasian and American black PSS patients had HLA-DQB1*0301(DQw7), *0302(DQw8), or *0303(DQw9), which all constitute the broad specificity HLA-DQw3. Examination of the amino acid sequences of these alleles (Table VI) showed extensive amino acid sequence homology. The presence of HLA-DQB1*0601–*0604(DQw6) or DQw4 in all antitopo I–positive patients who were negative for HLA-DQw3, as well as the decreased frequencies of HLA-DQw5 and DQw2.1, suggests an amino acid sequence shared by HLA-DQB1 alleles comprising HLA-DQw3, DQw4, and DQw6 specificities but not found in those

Table IV. HLA-DRB1 Frequencies in Autoantibody Subsets of Progressive Systemic Sclerosis in American Blacks

HLA-DRB1	Antitopo I (n = 11)	Other PSS (n = 18)	Controls (n = 88)
*0102(DR1)	1 (9%)	2 (11%)	8 (9%)
*1501(DR2)	0 (0%)	1 (5%)	3 (3%)
*1502(DR2)	0 (0%)	1 (5%)	0 (0%)
*1503(DR2)	2 (18%)	6 (33%)	16 (18%)
*0301(DR3)	0 (0%)	3 (17%)	9 (10%)
*0302(DR3)	1 (9%)	2 (11%)	12 (14%)
*0401(DR4)	1 (9%)	0 (0%)	3 (3%)
*0403(DR4)	1 (9%)	0 (0%)	1 (1%)
*0407(DR4)	1 (9%)	0 (0%)	1 (1%)
*1101–*1104(DR5)	5 (45%)	3 (17%)	20 (23%)
*1101(DR5)	3 (27%)	0 (0%)	11 (13%)
*1102(DR5)	1 (9%)	3 (17%)	6 (7%)
*1104(DR5)	1 (9%)	0 (0%)	2 (2%)
*1201(DR5)	0 (0%)	1 (5%)	3 (3%)
*1301(DRw6)	1 (9%)	2 (11%)	11 (13%)
*1302(DRw6)	0 (0%)	3 (17%)	4 (5%)
*1303(DRw6)	1 (9%)	0 (0%)	5 (6%)
*1402(DRw6)	0 (0%)	1 (5%)	4 (5%)
*0701(DR7)	1 (9%)	2 (11%)	22 (25%)
*0802(DRw8)	2 (18%)	0 (0%)	3 (3%)
*0803(DRw8)	1 (9%)	0 (0%)	2 (2%)
*0804(DRw8)	1 (9%)	4 (22%)	6 (7%)
*0901(DR9)	0 (0%)	2 (11%)	0 (0%)
*1001(DRw10)	0 (0%)	1 (5%)	4 (5%)

‡ HLA-DR specificities composed of the respective HLA-DRB1 alleles are shown in parentheses. HLA-DRB1*0101, *0103, *1601, *1602, *0402, *0404–*0406, *0408–*0411, *1103, *1401, and *0801 were found in none of the patients and their frequencies are thus not shown.

Table V. HLA-DQA1 and DQB1 Frequencies in Autoantibody Subsets of Progressive Systemic Sclerosis in American Blacks

	Antitopo I (n = 11)	Other PSS (n = 18)	Controls (n = 88)
HLA-DQA1			
*0101	3 (27%)	3 (17%)	28 (32%)
*0102	3 (27%)	8 (44%)	26 (30%)
*0103	0 (0%)	2 (11%)	16 (18%)
*0201	1 (9%)	3 (17%)	24 (27%)
*03	4 (36%)	2 (11%)	13 (15%)
*0401	4 (36%)	6 (33%)	19 (22%)
*0501	5 (45%)	8 (44%)	33 (38%)
HLA-DQB1			
*0501(DQw5/DQw1)‡	4 (36%)	3 (17%)	21 (24%)
*0601–*0604(DQw6/DQw1)‡	3 (27%)	11 (61%)	40 (45%)
*0601	1 (9%)	1 (6%)	4 (5%)
*0602	1 (9%)	9 (50%)	16 (18%)
*0604	1 (9%)	3 (17%)	7 (8%)
*0201(DQw2)‡	2 (18%)	7 (39%)	30 (34%)
*0301(DQw7/DQw3)‡	9 (82%)§‖	7 (39%)	29 (33%)
*0303(DQw9/DQw3)‡	0 (0%)	1 (5%)	3 (3%)
*0402(DQw4)‡	3 (27%)	3 (17%)	14 (16%)

‡ Numbers in parentheses refer to specific/broad HLA-DQ specificities composed of the DQB1 allele shown. No patients had HLA-DQA1*0601, DQB1*0502, DQB1*0503, DQB1*0603, or DQB1*0302 (not shown). § P = 0.05, OR = 7 (uncorrected) compared with other PSS patients. ‖ pc = 0.02, OR = 9.2 compared with controls.

comprising DQw5 or DQw2. Several possible candidate sequences thereof derived from Table VI are shown in Table VII. One, a tyrosine residue at position 30 (tyr-30), is shared by HLA-DQB1*0402, *0601, *0602, as well as by DQB1*0301, *0302, and *0303. An HLA-DQB1 allele bearing tyr-30 was present in 100% of antitopo I–positive Caucasians compared with 66% of other PSS patients and 70% of Caucasian controls (pc < 0.01 in both cases). HLA-DQB1-tyr-30 was likewise found in 100% of American black antitopo I–positive PSS patients. The allelic frequency of HLA-DQB1-tyr-30 was also slightly more frequent in American blacks with antitopo I (80%) versus black PSS patients without antitopo I (61%), although this difference was not statistically significant, perhaps because of the small numbers studied.

Alternatively, other polymorphic amino acids residues are shared by these HLA-DQB1 alleles, including methionine in position 14, alanine in position 38, and threonine in position 77. Each of these occurred in 100% of the antitopo I–positive Caucasians (Table VII) and blacks. Unlike tyrosine at position 30, however, none of these residues was statistically significantly increased in antitopo I–positive patients compared with other PSS patients and normal controls. Nonetheless, a role for one or more of these residues in susceptibility to this autoimmune response cannot be excluded.

The association of antitopo I with HLA-DQB1 rather than HLA-DRB1 or DRB3 alleles is further underscored by examining antitopo I–positive versus –negative PSS patients and controls who were negative for HLA-DR5(DRw11). The allelic frequency of HLA-DQB1*0301(DQw7) was 21% in 17 HLA-DR5(DRw11)–negative, antitopo I–positive Caucasian and American black PSS patients compared with 9% in antitopo I–negative PSS patients and 10% in race-matched controls (P = NS for both groups). The allelic frequency of the broader specificity HLA-DQw3 was 47% in HLA-DR5(DRw11)-negative Caucasian and American black antitopo I–positive PSS patients compared with 20% in antitopo I–negative patients (P = NS) and 25% in controls (P = 0.04). On the other hand, HLA-DQB1-tyr-30-bearing alleles occurred in 68% of 34 HLA-DR5(DRw11)–negative, antitopo I–positive patient haplotypes compared with 40% of 142 antitopo I–negative PSS patient haplotypes (P = 0.03) and 60% of control haplotypes (P = NS). Evaluation of a possible HLA-DR5(DRw11) association independent of HLA-DQB1*0301(DQw7), DQw3, or DQB1-tyr-30 was complicated by the finding that only two PSS patients had an HLA-DR5–bearing allele not associated with HLA-DQB1*0301(DQw7), one with HLA-DQB1*0602(DQw6) (antitopo I positive) and another with HLA-DQB1*0201 (DQw2) (antitopo I negative).

That HLA-DQ and not DR genes represent the primary MHC class II association with the antitopo I response is further suggested by the presence of HLA-DQB1*0301(DQw7) in all three native American Choctaw PSS patients found to have antitopo I. Two were homozygous for HLA-DQB1*0301

Table VI. DQ Beta First Domain Amino Acid Sequences

	10	20	30	40	50	60	70	80	90	
Not associated with antitopo I										
	RDSPEDFVYQ	FKGLCYFTNG	TERVRGVTRH	IYNREEYVRF	DSDVGVYRAV	TPQGRPVAEY	WNSQKEVLEG	ARASVDRVCR	HNYEVAYRGI	LQRR
DQw5;DQB1*0501	----------	---M------	-----L-S-S	------I---	-----EF---	-LL-L-A---	-----DI--R	K--A------	---QLEL-TT	----
DQw2;DQB1*0201	----------	----------	----------	----------	----------	----------	----------	----------	----------	----
DQw5;DQB1*0502	----------	----------	----------	----------	----------	------S---	----------	----------	----------	----
DQw5;DQB1*0503			----------	----------	----------	------D---	----------	----------		
Possibly associated with Antitopo I (DQB1 alleles found in HLA-DQw3-negative, antitopo I-positive PSS patients)										
DQw4;DQB1*0402	--------F-	---M------	-----G--Y	------A--	----------	--L--LD---	-----DI--E	D-----T--	---QLEL-TT	----
DQw6;DQB1*0601	--P-----L-	--AM------	-----Y--Y	-----D---	----------	------D---	-----DI--R	T--EL-T--	------F---	----
DQw6;DQB1*0602	--------F-	---M------	-----L--Y	------A--	----------	------D---	----------	T--EL-T--	------F---	----
DQw6;DQB1*0603			--L------	------A--	----------	------D---	----------	T--EL-T-		
DQw6;DQB1*0604	----------	---M------	-----L----	------A--	----------	----------	------R---	T--EL-T--	-----G----	----
Associated with antitopo I										
DQw7;DQB1*0301	----------	--AM------	-----Y--Y	---------A--	----E-----	--L-P-D---	----------	R T--EL-T--	---QLEL-TT	----
DQw8;DQB1*0302	----------	---M------	-----L--Y	---------A--	----------	--L-P-A---	----------	R T--EL-T--	---QLEL-TT	----
DQw9;DQB1*0303	----------	---M------	-----L--Y	---------A--	----------	--L-P-D---	----------	R T--EL-T--	---QLEL-TT	----

(DQw7) and the third had this allele and DQw4. Only one of these had HLA-DR5(DRw11). Instead, two had HLA-DQB1*0301(DQw7) associated with the rare DRB1*1602 subtype of HLA-DR2.

Discussion

Our data suggest that the broad specificity HLA-DQw3 is most strongly associated with the antitopo I response. HLA-DQw3 comprises HLA-DQB1*0301(DQw7) (linked to HLA-DR5(DRw11) as well as other DR specificities), DQw8 (associated with HLA-DR4), and DQw9 (associated with HLA-DR9 and occasionally HLA-DR7) (56). The HLA-DQB1 alleles comprising HLA-DQw3 share striking sequence homology (Table VI). All remaining HLA-DQw3–negative antitopo I–positive PSS patients had HLA-DQw6 (specifically HLA-DQB1*0602), which is found on HLA-DR2(DRB1*1501) haplotypes (among others), or DQw4, associated in Caucasians with HLA-DRw8(DRB1*0801, *0802) and in blacks with HLA-DRw8 and HLA-DR3(DRB1*0302). HLA-DQw3 (DQB1*0301, *0302, and *0303), DQw6 (DQB1*0602), and DQw4 (DQB1*0402) share specific amino acid residues not found in the same position on other HLA-DQB1 alleles. In the second hypervariable region, for example, there is a polar uncharged tyrosine residue at position 30 (tyr-30), as opposed to a polar uncharged serine or a positively charged histidine. At position 38 there is a hydrophobic alanine (ala-38) as opposed to a hydrophobic valine residue. In the third hypervariable region there is a polar uncharged threonine at position 77 (thr-77) as opposed to a positively charged arginine. Analysis of the frequencies of alleles bearing these residues suggests the strongest association of these to be with tyr-30 alleles. Thus although HLA-DQw3 (especially the DQB1*0301(DQw7) subtype thereof) is most strongly associated with the antitopo I response, the ubiquity of tyr-30–bearing DQB1 alleles in HLA-DQw3 negatives suggests that HLA-DQB1-tyr-30 may form the minimum requirement for the antitopo I response. We cannot exclude the possibility, however, that one or more of the other shared polymorphic sequences in DQB1 alleles may also play important roles.

These data, then, allow a reinterpretation of previous apparently conflicting reports of serologically defined HLA class II allele frequencies in PSS. Differences in autoantibody frequencies in the different patient groups reported could explain at least in part some of these discrepancies. For example, reports of an increased frequency of HLA-DR1 (13–16) could be from patient populations with a higher frequency of ACA, or associations with HLA-DR3 (and perhaps even C4 null alleles, which are linked to HLA-DR3 [11, 17, 19]) could reflect a higher frequency of anti-PM-Scl. Other reports showing associations with HLA-DR5 (16, 18–20) could be explained by a higher proportion of patients with antitopo I and/or ACA.

Apparent discrepancies of HLA-DR associations with autoantibody subsets of PSS also could be better explained by their linkage disequilibrium with certain HLA-DQ alleles, which in fact may be the primary associations. For example, different reports have linked the ACA response to HLA-DR1 alone (27), DR1, DR4, and/or DRw8 (36), and DR5(DRw11) (20). These HLA-DR specificities share no common unique amino acid sequences (57). However, DNA analyses from our own laboratory (37) have shown stronger

Table VII. Phenotypic and Genotypic Frequencies of HLA-DQB1 Amino Acid Residues in Caucasian Progressive Systemic Sclerosis Patients

HLA-DQB1	Antitopo*	ACA*	Other PSS	Controls
Phenotypic	(n = 21)	(n = 47)	(n = 65)	(n = 200)
Meth 14	21 (100%)	44 (93%)	65 (100%)	196 (98%)
Tyr 30	21 (100%)‡§	38 (80%)	43 (66%)	140 (70%)
Ala 38	21 (100%)	39 (84%)	50 (77%)	162 (81%)
Thr 77	21 (100%)	40 (86%)	50 (77%)	164 (82%)
TRAELDT 71-77	20 (95%)	39 (84%)	46 (71%)	164 (82%)
Genotypic (allelic)	(n = 42)	(n = 94)	(n = 130)	(n = 400)
Meth 14	37 (88%)	66 (70%)	106 (82%)	328 (82%)
Tyr 30	31 (74%)‖¶	55 (58%)	55 (42%)	184 (46%)
Ala 38	31 (74%)	57 (61%)	67 (52%)	236 (59%)
Thr 77	31 (74%)	60 (64%)	68 (52%)	240 (60%)
TRAELDT 71-77	32 (76%)**	55 (58%)	60 (46%)	228 (57%)

* Including one patient with both ACA and antitopo, associated with HLA-DR3 (DRw17), DQw2.1, and HLA-DRw8, DQw4 specificities and is included in both columns. ‡ pc = 0.007 compared with "other PSS" patients. All P values are corrected by multiplying by the five variables examined. § pc = 0.008 compared with controls. ‖ pc = 0.0035 compared with other PSS patients. ¶ pc = 0.005 compared with controls. ** pc = 0.005 compared with other PSS patients.

associations of ACA with HLA-DQB1 alleles in linkage disequilibrium with these HLA-DR antigens: HLA-DQw5 (linked to HLA-DR1); HLA-DQB1*0301(DQw7) (found on most HLA-DR5 and many DR4-bearing haplotypes) (54), and HLA-DQw4 (found on most Caucasian HLA-DRw8 haplotypes). These HLA-DQ specificities have in common the presence of a polar amino acid residue at position 26 of the HLA-DQB1 outermost domain and have been found in all of the ACA-positive patients studied thus far (37).

Similarly, for the antitopo I response, the previous serologic HLA associations have been with HLA-DR2 (25) and DR5 (27, 36), which share no unique amino acid sequences (57); however, HLA-DQB1*0602(DQw6) and HLA-DQB1*0301 (DQw7), which are in linkage disequilibrium with these DR specificities, respectively (55), share sequence homologies in their second and third hypervariable regions of the HLA-DQB1 outermost domain. These uniquely shared amino acid residues in these regions were found in all of the antitopo I–positive PSS patients.

The only patient with both ACA and antitopo I antibodies in this study was found to have HLA-DQw4, a specificity that possesses a polar amino acid at DQB1 position 26 and a tyrosine at DQB1 position 30. Interestingly, HLA-DQB1*0301 (DQw7) also possesses both of these potentially relevant DQB1 sequences and is increased in frequency in both ACA-positive and antitopo I–positive PSS patients, yet both autoantibodies rarely coexist in the same patient (27). An explanation for this near mutual exclusivity of autoantibody expression is not apparent, however, it suggests a role for non-HLA factors, either genetic or environmental, in triggering these autoimmune responses.

Still, there were some PSS patients who had HLA-DQB-tyr-30 alleles and did not express antitopo I, although the converse was not found. One possible reason could be that the serum level of antitopo I antibodies they expressed were below those detectable by our immunodiffusion and immunoblotting assays. Alternatively, these patients may have lacked the appropriate T cell receptor genes to whose products the HLA-DQ heterodimers could present the processed antitopo I autoantigen. Another alternative would be that the HLA associations are better explained by clinical subsets of PSS, which are themselves associated with antitopo antibodies. This was not found, however, in this study.

Finally, these data regarding antitopo I antibodies, as well as our previous studies of ACA, suggest that scleroderma, similar to systemic lupus erythematosus (58), can be viewed as a composite disease of several HLA-DQ–associated autoantibody (and in turn clinical) subsets. Additional direct evidence that HLA-DQ mediates these autoimmune responses is needed, as well as studies to determine whether these highly specific autoantibodies participate in the pathogenesis of PSS.

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