Human Leukocyte Antigen-DRB1 Position 11 Residues Are a Common Protective Marker for Sarcoidosis

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Genetic factors, in particular human leukocyte antigens (HLAs) are important determinants of susceptibility to sarcoidosis, a chronic granulomatous disease of undetermined etiology. To clarify the role of HLA in sarcoidosis we determined HLA-DR and -DQ alleles in case-control samples from three European populations (United Kingdom, Czech, and Polish) and compared these results with those published for three additional populations (Italian, Japanese, and Scandinavian) to determine whether the HLA-DR and/or -DQ alleles act as ethnic-dependent, or ethnic-independent modifiers of disease risk. Although variations were apparent in the alleles associated with susceptibility, reductions in the frequency of alleles associated with protection were remarkably consistent in the six populations. Previously detected associations between single-nucleotide polymorphisms at the TAP2 locus and sarcoidosis were shown to be due to linkage disequilibrium with the HLA-DR locus. The protective HLA-DR alleles, which encode the DR1 and DR4 antigens, were found to share characteristic small hydrophobic residues at position 11, which were replaced by small hydrophilic residues in the remaining, nonprotective, HLA-DR alleles. This residue position is within a pocket of the HLA-DR complex antigen binding groove (designated P6), where it is the only variable amino acid and therefore determines the peptide binding preferences of this pocket. A highly significant reduction in the frequency of individuals carrying HLA-DR alleles with a hydrophobic residue at position 11 was observed in the sarcoidosis cases in the three populations we examined. This suggests this HLA-DR residue is an important protective marker in sarcoidosis.

Sarcoidosis, a disease predominantly affecting young adults, is the most common diffuse lung disease, with a population prevalence of around 1:4,000 in the United Kingdom (UK). Increased disease risk among patients' families and varying racial disease incidences suggest that genetic factors are important determinants of susceptibility to this chronic granulomatous disease of undetermined etiology (1). In sarcoidosis, CD4+ T cells accumulate at the site of active disease, where they are believed to interact via their T-cell receptor for antigen with human leukocyte antigen (HLA) molecules expressed by antigen-presenting cells (APCs). Expression of HLA-DR on alveolar macrophages from pulmonary sarcoidosis patients has been shown to be increased (2), as has the antigen-presenting capacity of these cells (3). These findings suggest that in sarcoidosis, antigen presentation by APCs occurs via the HLA class II restricted pathway, and that this process is likely to be central to the initiation and maintenance of the granulomatous inflammation observed.

Several studies have examined major histocompatibility complex (MHC) class II alleles in sarcoidosis, but have not identified strong associations consistently (4, 5) possibly reflecting small sample sizes, involvement of nearby "nonclassical" genes within the MHC region, and ethnic, or clinical, heterogeneity in subjects studied. Use of multiple clearly defined ethnic populations may therefore be a useful strategy when performing studies of this disease. We previously examined whether specific alleles or allelic motifs (residues common to multiple alleles) at the HLA-DPB1 locus contribute to sarcoidosis susceptibility in a case-controlled study of the UK population (6). No evidence of associations with specific alleles was detected at this locus, in agreement with findings in African-American patients (7). No allelic motif associations were observed in the UK patients, although the Val36- and Asp55-positive HLA-DPB1 alleles were associated with increased disease risk in the previous African-American patient study. In our previous study, associations were detected between sarcoidosis and single-nucleotide polymorphisms (SNPs) in the TAP2 antigen-processing gene, located within the MHC class II region, in case-control samples from both the UK and Polish populations. SNPs at the nearby TAP1 gene showed no association with disease. The lack of association between polymorphic loci located centromeric to the TAP2 gene (i.e., the HLA-DP and TAP1 loci) suggests that the TAP2 gene may represent a boundary of the MHC region involved in sarcoidosis susceptibility. In the present study, we examined allelic variation at the HLA-DR and -DQ loci, which are telomeric to the TAP2 loci, to clarify further the exact role this MHC region plays in determining susceptibility to sarcoidosis. An association between a particular HLA allele and disease across different ethnic groups lends support to the direct participation of that locus in disease susceptibility (8). To identify whether any relationships between alleles and disease in sarcoidosis are consistent across ethnic boundaries, we studied three groups of case-control samples from the UK Caucasian, Polish, and Czech populations.

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Abbreviations: χ-square, χ^2; 95% confidence interval, 95% CI; degrees of freedom, d.f.; human leukocyte antigen, HLA; hydrophobic side chain, HS; major histocompatibility complex, MHC; nonhydrophobic side chain, NHS; odds ratio, OR; polymerase chain reaction, PCR; single-nucleotide polymorphism, SNP.

TABLE 1
Demographic data on UK, Polish, and Czech sarcoidosis patients

	UK Sarcoid			Polish Sarcoid			Czech Sarcoid		
	F	M	Total	F	M	Total	F	M	Total
Number (*n*)	89	100	189	48	39	87	38	31	69
Age at presentation (yr)									
Mean	40	39	39	53	44	49	48	42	45
Standard error of the mean	10	8	9	9	7	9	9	8	11
Smoker?									
Yes	3	5	8	3	7	10	2	5	7
No	84	97	181	45	32	77	36	26	62

Materials and Methods

Subjects

Patients at the three participating study centers were defined using the ATS/ERS/WASOG Statement on Sarcoidosis (9). All patients were diagnosed on the basis of noncaseating granulomas in a tissue biopsy and had clinical features of pulmonary sarcoidosis. UK cases (n = 189) were recruited from the Royal Brompton Hospital, London, UK; Czech cases (n = 69) from the Olomouc University Hospital, Olomouc, Czech Republic; and Polish cases (n = 87) from the Institute of Tubercle and Lung Diseases, Warsaw, Poland. Unrelated, ethnically matched controls were Caucasian cadaveric organ donors from the Oxford Tissue Typing Centre (n = 288), Oxford, UK; Czech nationality blood donors from the Olomouc region (n = 158); and healthy medical students from the Medical University of Gdansk, Gdansk, Poland (n = 133). Patient demographics, including age at presentation, sex, and smoking history are outlined in Table 1. Part of the UK case population (n = 117) and all of the UK controls and Polish cases and controls were included in our previous study of different genes (6). Genotype data on the TAP genes from our previous study is referred to for linkage. Project approval was granted by the local ethics committee at each of the three centers.

HLA-DR and -DQ Genotyping

Genomic DNA was isolated from whole-blood samples according to Miller and colleagues (10). A 48-reaction sequence-specific primer (SSP) polymerase chain reaction (PCR) assay was used to determine the HLA-DR and -DQ genotypes for each individual studied. PCR reaction mixture components, cycling parameters, and the detection of PCR products by agarose gel electrophoresis were as described previously (11).

Comparison of HLA Results with Published Data

To determine whether any alleles represent ethnic-independent susceptibility or protection factors, the results for the populations in this study were compared with those published previously for case-control studies from Scandinavia, Italy, and Japan (12–14). Studies used for comparison were chosen on the basis of size (all three studies examined in excess of 100 cases, and 250 matched controls) and provision of a medium resolution HLA-DR phenotype frequency breakdown. In the case of the Japanese population, several HLA-DR studies have been described; the study chosen for comparison represented the largest of these. In the previously published studies used in the comparisons, HLA data was obtained using both serologic and SSP-PCR methodology. These methodologies conformed to the World Health Organization (WHO)–defined standards for HLA nomenclature and typing used here.

After comparison of the results, HLA-DR alleles were classed as "susceptible" or "protective" if the phenotype frequency was increased or decreased, respectively, in patients compared with matched controls.

We also compared the results of HLA-DR and -DQ genotyping with our previous TAP2 genotyping results for the UK and Polish patient groups (6) to determine whether associations observed at the HLA-DR and -DQ loci in the present study occurred independently of those observed previously at the TAP2 locus.

HLA-DRB1 Protein Sequence Comparisons

Protein sequences of HLA-DRB1 alleles were obtained from the IMGT/HLA Database (15). The relative positions of HLA-DRB1

TABLE 2
Phenotype frequencies (%) of HLA-DR and -DQ alleles in sarcoidosis cases and controls from three European populations

	UK		Polish		Czech	
Allele	Cases (n = 189)	Controls (n = 288)	Cases (n = 87)	Controls (n = 133)	Cases (n = 69)	Controls (n = 158)
DRB1*01	11.2	19.3*	10.3	29.3*	10.1	16.5
DRB1*04	25.5	35.0*	23	21.1	15.9	17.1
DRB1*07	19.7	26.7	23	25.6	10.1	25.9*
DRB1*08	3.2	4.8	12.6	6.8	7.2	3.2
DRB1*09	1.6	2.5	2.3	0.8	1.4	0.6
DRB1*10	2.7	2.3	0	2.3	0	0
DRB1*11	17.6	12.5	31	23.3	20.3	22.2
DRB1*12	10.1	2.7*	2.3	5.3	2.9	4.4
DRB1*13	17.6	19.3	19.5	18.8	26.1	18.3
DRB1*14	16.5	4.7*	5.7	2.3	15.9	6.3*
DRB1*15	38.8	27.9*	33.3	18.8*	34.8	29.7
DRB1*16	0.5	1.2	4.6	7.5	7.2	12
DRB1*17	25	26.1	21.8	24.1	33.3	27.2
DQB1*02	37.8	43.1	36.8	40.6	47.8	27.8*
DQB1*04	2.7	5.9	12.6	5.3	8.7	2.5*
DQB1*05	29.3	32.3	20.7	41.4*	33.3	25.9
DQB1*0601	0	0.4	0	0	0	0
DQB1*0602	38.6	23.3*	32.2	18.0*	39.1	30.4
DQB1*0603-9	3.7	16.6*	11.5	3.0*	11.6	5.7
DQB1*0301/4	36.2	26.4*	36.8	38.3	26.1	25.9
DQB1*0302	12.8	18.4	18.4	14.3	13	8.9
DQB1*03032	6.4	3.1	9.2	3	1.4	3.1

*Significant differences in phenotype frequency between cases and controls. Individual P values and confidence intervals for these differences are detailed in RESULTS.

TABLE 3
Linkage disequilibrium between HLA-DRB1 and TAP2 in UK and Polish cases studied

		DRB1*01+	DRB1*01−	ΔS	χ^2	P*
UK cases (n = 132)	TAP2*0201+	11	29	0.61	14.8	0.0001
	TAP2*0201−	4	88			
Polish cases (n = 87)	TAP2*0201+	7	27	0.63	6.3	0.01
	TAP2*0201−	2	51			

*Fisher's Exact test used for the Polish comparison.

residues forming pockets within the peptide binding groove of the HLA-DR complex were identified from crystallography data for a HLA-DR peptide complex (16).

Statistical Analysis

Statistical analysis of the HLA frequency data for the three populations studied used a χ-square (χ^2) contingency table analysis with the appropriate number of degrees of freedom. Significant differences between cases and controls were determined by a P value of less than 0.05. Combined analysis of data from the three centers used the Mantel–Haenszel test for association, with stratification according to ethnic group, after testing to exclude heterogeneity between the centers. Analysis of linkage disequilibrium and standardized Δ values (ΔS) for associations between TAP2 and HLA-DR alleles were as previously described (17).

In case-control studies where the disease is rare in the general population from which cases and controls are selected, such as sarcoidosis, the odds ratio (OR) may be used as an approximation of the relative risk. ORs and 95% confidence intervals (95%CIs) were calculated for the phenotypes and genotypes found to differ significantly. Our case-control sample sizes allowed us to detect a difference of 10% in phenotype or genotype frequency with 93.1% power (P = 0.05) in the UK population, with 63% power in the Polish population (P = 0.05), and with 61% power in the Czech population (P = 0.05). If an ethnic-independent genetic effect was present, the combined patients and controls would, theoretically, detect a 10% increase in exposure within the patient group with 98.5% power (P = 0.01). Statistical tests were performed using the statistical program EpiInfo6 (WHO, Geneva, Switzerland/Centers for Disease Control, Atlanta, GA).

Results

HLA-DRB1 and -DQB1 Phenotypes in Three European Case-Control Populations

The HLA-DRB1 and -DQB1 phenotype results for the UK, Polish, and Czech cases and controls are given in Table 2.

In the UK population, an increased risk of disease was associated with the HLA-DRB1*12 (P = 0.001, OR = 3.93, 95%CI 1.59 to 10.03), -DRB1*14 (P = 0.00004, OR = 3.86, 95%CI 1.91 to 7.9), and -DRB1*15 (P = 0.02, OR = 1.65, 95%CI 1.10 to 2.49) alleles at the HLA-DR locus; and with the HLA-DQB1*0602 (P = 0.0003, OR = 2.09, 95%CI 1.37 to 3.18) and -DQB1*0301/4 (P = 0.03, OR = 1.58, 95%CI 1.04 to 2.4) alleles at the HLA-DQ locus. A reduced risk of disease was found to be associated with the HLA-DRB1*01 (P = 0.02, OR = 0.52, 95%CI 0.29 to 0.92), -DRB1*04 (P = 0.04, OR = 0.63, 95%CI 0.41 to 0.97), and HLA-DQB1*0603-9 (P = 0.00001, OR = 0.19, 95%CI 0.08 to 0.45) alleles in the UK population.

In the Polish population, HLA-DRB1*15 (P = 0.02, OR = 2.16 95%CI 1.11 to 4.22) and -DQB1*0602 (P = 0.02, OR = 2.20, 95%CI 1.12 to 4.25) were associated with increased risk of disease, and HLA-DRB1*01 (P = 0.002, OR = 0.28, 95%CI 0.12 to 0.64), -DQB1*05 (P = 0.002, OR = 0.37, 95%CI 0.19 to 0.72), and -DQB1*0603-9 (P = 0.01, OR = 4.19, 95%CI 1.15 to 16.49) were associated with a reduced risk of disease. In the Czech population, an increased disease risk was associated with the HLA-DRB1*14 (P = 0.02, OR = 2.8, 95%CI 1.04 to 7.6), -DQB1*02 (P = 0.003, OR = 2.4, 95%CI 1.3 to 4.5), and -DQB1*04 (P = 0.04, OR = 3.67, 95%CI 0.9 to 16.1) alleles. The HLA-DR7 allele was associated with a reduced risk of disease (P = 0.007, OR = 0.32, 95%CI 0.12 to 0.8) in the Czech population.

The close proximity of the TAP2 gene, where we previously described associations in sarcoidosis (6), to the HLA-DR and -DQ genes suggested that associations at the two loci may not be independent, due to linkage disequilibrium between the loci. Data from a study of 115 homozygous typing cell lines has suggested that HLA-DRB1*01 may be in linkage disequilibrium with the TAP2*0201 allele (which comprises three TAP2 variant residues Val379-

TABLE 4
Consistently protective HLA-DRB1 alleles in sarcoidosis

		Phenotype Frequency		OR (95%CI)*	
Population	n	DRB1*01	DRB1*04	DRB1*01	DRB1*04
UK cases	189	0.112	0.255	0.52 (0.29–0.92)	0.63 (0.41–0.97)
UK controls	288	0.193	0.350		
Czech cases	69	0.101	0.159	0.41 (0.14–1.11)	0.92 (0.40–2.10)
Czech controls	158	0.165	0.171		
Polish cases	87	0.103	0.230	0.28 (0.12–0.64)	1.02 (0.51–2.05)
Polish controls	133	0.293	0.211		
Scandinavian cases (12)	122	0.130	0.210	0.69 (0.35–1.32)	0.46 (0.27–0.78)
Scandinavian controls	250	0.180	0.370		
Japanese cases (14)	113	0.009	0.336	0.08 (0–0.56)	0.66 (0.42–1.03)
Japanese controls	478	0.098	0.435		
Italian cases (13)	107	0.065	0.074	0.40 (0.16–0.92)	0.52 (0.22–1.16)
Italian controls	510	0.165	0.135		

*ORs for the HLA-DRB1*01 and -DRB1*04 alleles in each population were calculated on the risk conferred by the presence of one or more copies of that allele.

Ala565-Ala665) (18). This association was confirmed in our UK and Polish patient populations (Table 3). The frequency of the TAP2 variants Val379 and Ala665 were found to be significantly reduced in the previous UK and Polish population study, and these reductions correlate with the reductions in the HLA-DRB1*01 frequency seen in the present study.

Comparison of HLA Phenotype Results with Published Data

Results for the three populations in this study were compared with results previously published for Scandinavian, Italian, and Japanese case-control studies. The "susceptible" alleles were found to vary between populations, but reductions in the frequency of "protective" alleles, encoding the DR1 and DR4 antigens, were remarkably consistent in the six populations (Table 4).

Sequence Comparison of "Susceptible" and "Protective" Alleles

To identify common features exclusive to either the "protective" (HLA-DRB1*01 and -DRB1*04) or the "susceptible" (HLA-DRB1*08, -DRB1*09, DRB1*12, -DRB1*14, -DRB1*15, and -DRB1*17) allele groups, protein sequences of the HLA-DRB1 alleles were compared. One such residue was identified: in the "protective" alleles, at position 11, a valine or leucine was present, whereas in the "susceptible" alleles a glycine, serine, or proline was found at this position. Structurally, valine and leucine are small residues and have bulky, highly hydrophobic, aliphatic side chains, whereas serine, glycine, and proline are small and do not have highly hydrophobic side chains. The remaining HLA-DRB1 alleles were also found to contain small, hydrophilic residues at position 11. The peptide binding specificity of HLA-DR is determined by clusters of polymorphic residues which form nine pockets in the HLA-DR groove (designated P1 through P9) (16). The variable residues confer distinct chemical and size characteristics to each pocket in different HLA-DRB1 alleles (19). Within the P6 cluster, HLA-DRB1 position 11 is the only variable residue and therefore determines the binding preferences of this pocket.

Influence of HLA-DRB1 Position 11 Side-Chain Properties on Susceptibility to Sarcoidosis

To determine the significance of hydrophobic or hydrophilic side-chain residues at position 11 of the HLA-DRB1 peptide in sarcoidosis, the HLA-DR results were reclassified into "hydrophobic side-chain" (HS) and "nonhydrophobic side-chain" (NHS) alleles based on the residue 11 side-chain properties. These results were then compared among our three study populations (Table 5). Analysis of the reclassified data showed a significant negative association between sarcoidosis and DRB1 genotypes and alleles in both the UK ($\chi^2 = 20.3$, degrees of freedom (d.f.) = 2, $P = 0.00004$, and $\chi^2 = 20.8$, d.f. = 1, $P = 0.000005$, respectively) and Polish ($\chi^2 = 7.7$, d.f. = 2, $P = 0.02$, and $\chi^2 = 5.1$, d.f. = 1, $P = 0.02$, respectively) samples. The variance in genotypic distribution appeared attributable to a reduction in the HLA-DRB1 HS alleles in the sarcoidosis cases (UK: $P = 0.0001$, OR = 0.46, 95%CI = 0.31 to 0.69; Polish: $P = 0.008$, OR = 0.46, 95%CI = 0.25 to 0.85). In the Czech sample, a similiar reduction was detected but did not reach significance when tested (OR = 0.79, 95%CI = 0.40 to 1.55). Combining the three study samples in a stratified analysis showed that the sarcoidosis cases had a highly significant reduction in the HLA-DRB1 HS alleles ($\chi^2 = 24.6$, d.f. = 2, $P = 0.0000007$). This yielded a Mantel–Haenszel weighted OR of 0.56 (0.44 to 0.7), with no evidence of heterogeneity between samples ($P = 0.33$).

Discussion

In this study we examined HLA-DR and -DQ allele distributions in three European case-control samples to clarify the role that the MHC class II region plays in determining susceptibility to sarcoidosis. Our use of the words "population," "ethnic," and "ethnic boundaries" in referring to these three groups is intended to convey that the three case-control samples chosen have genetic backgrounds that are distinct from each other. That the UK, Polish, and Czech samples are in fact genetically different is apparent from the HLA distribution in the control groups from each country that show clear differences, in agreement with published HLA data for the English, Polish, and Czech populations which also show differences in HLA distribution between these groups (15). Results for the three populations examined (UK, Polish, and Czech) were further compared with those published for three additional populations (Italian, Japanese, and Scandinavian) to determine whether the HLA-DR and/or -DQ alleles act as ethnic-dependent, or ethnic-independent modifiers of disease risk. Although variations were apparent in the alleles associated with susceptibility, reductions in the frequency of alleles associated with protection were remarkably consistent in the six populations. These protective alleles, which encode the DR1 and DR4 antigens, were found to share characteristic small hydrophobic residues at position 11, which were replaced by small hydrophilic residues in the remaining, non-

TABLE 5

HLA-DRB1 hydrophobic position 11 alleles in sarcoidosis

	Genotypes			Allele		OR
	HS HS	HS NHS	NHS NHS	HS	NHS	(95%CI)*
UK cases	8	61	120	77	301	
	(0.04)	(0.32)	(0.63)	(0.20)	(0.80)	0.46
UK controls	34	128	126	190	374	(0.31–0.69)
	(0.12)	(0.44)	(0.44)	(0.34)	(0.66)	
Czech cases	1	18	50	20	118	
	(0.01)	(0.26)	(0.72)	(0.14)	(0.86)	0.79
Czech controls	10	38	110	58	258	(0.4–1.55)
	(0.06)	(0.24)	(0.70)	(0.18)	(0.82)	
Polish cases	7	18	62	32	142	
	(0.08)	(0.21)	(0.71)	(0.18)	(0.82)	0.46
Polish controls	12	50	71	74	192	(0.25–0.85)
	(0.09)	(0.38)	(0.53)	(0.28)	(0.72)	
Total cases	16	96	234	128	564	
	(0.04)	(0.28)	(0.68)	(0.18)	(0.82)	0.55
Total controls	56	210	307	322	824	(0.43–0.70)
	(0.10)	(0.37)	(0.54)	(0.28)	(0.72)	

*Numbers of cases and controls are as in Table 1. ORs for each population and for the total samples were calculated on the risk conferred by the presence of at least one of the HS alleles.

protective HLA-DR alleles. This residue position is within a pocket of the HLA-DR complex antigen binding groove (designated P6) (16), where it is the only variable amino acid and therefore determines the peptide binding preferences of this pocket (19). A highly significant reduction in the frequency of individuals carrying HLA-DR alleles with a hydrophobic residue at position 11 was observed in the sarcoidosis cases in the three populations we examined. This finding suggests that this HLA-DR residue is an important protective marker in sarcoidosis.

The results of this study indicate that MHC associations in sarcoidosis reflect involvement of HLA-DR rather than other class II loci. This is suggested by our finding that associations detected between SNPs at the TAP2 locus are due to linkage disequilibrium with the HLA-DR1 allele in both the UK and Polish populations. Involvement of the HLA-DQ locus is also unlikely, considering that the presence of a specific HLA-DR allele on a haplotype, rather than a HLA-DQ allele, appears to determine disease susceptibility. For example, the HLA-DQB1 allele -DQ5 is in strong linkage disequilibrium with the HLA-DRB1 alleles -DR1 and -DR14 in Caucasians. The HLA-DR1 and -DR14 alleles were found to be associated with disease "protection" and "susceptibility," respectively, in three European populations studied, suggesting that the HLA-DR allele rather than the -DQ allele is more influential in determining disease risk. Of interest also was the observation that HLA-DR14 and -15 were more common in the cases from the three European populations we examined, whereas the frequency of -DR17 was not altered. In the Scandinavian population, all three alleles were found to be associated with disease, with HLA-DR17 particularly associated with an acute disease course; whereas -DR14 and -DR15 were strongly associated with a chronic disease course (12). A similiar association between HLA-DR17 and acute disease has been described in German sarcoidosis cases (20). The absence of such a finding in our three study populations may reflect a higher proportion of patients with chronic disease in certain populations. The availability of disease course data on our study populations will enable us to further clarify this possibility in the future.

Our findings that certain HLA-DR alleles act as protective markers in sarcoidosis in a majority of populations examined suggest that this protective effect is ethnic-independent. In contrast, HLA-DR alleles associated with susceptibility appear to act in an ethnic-dependent manner, in agreement with previous findings (12, 14). For example, the HLA-DRB1*08 and -DRB1*09 alleles were relatively uncommon and not strongly associated with sarcoidosis in the three European populations we analysed, but were the most common alleles in Japanese patients and were strongly associated with disease (14). Conversely, HLA-DRB1*07 was found to be present at reduced frequency in the patient groups of the three populations examined in the current study (UK, Polish, and Czech), but at increased frequency in Scandinavian patients (12) and absent in Japanese patients and controls (14) examined previously. All of these alleles (HLA-DRB1*07, -DRB1*08, and -DRB1*09) carry hydrophilic residues at position 11, however their differing influences on disease susceptibility within specific populations suggest interactions between other elements within the HLA-DR complex in addition to the P6/residue 11 interaction proposed here. This is supported by the high frequency of individuals carrying HLA-DR alleles with hydrophilic residues at position 11 in our three control populations (88 to 94%). Absence of a hydrophobic residue at position 11 of HLA-DR alleles cannot therefore be considered as a discriminating feature of susceptibility associated alleles, inasmuch as such alleles are carried by a majority of the healthy population. In contrast, the presence of a hydrophobic residue at position 11 does discriminate protective alleles, and this is supported by the increased frequency of such alleles in the control groups from our three study populations (present in 30 to 55% of controls compared with 26 to 36% of cases).

The basis of a protective effect of hydrophobic residues at HLA-DRB1 position 11 in sarcoidosis is unknown, but may involve direct interaction with the peptide side chain or indirect means. Crystallography data indicates that the P6 pocket can accommodate water molecules concurrently with a side chain from a MHC-bound peptide (16). Because H_2O molecules can be important in binding, the presence of a hydrophobic position 11 side chain may alter the number/position of H_2O molecules in this pocket, thereby indirectly influencing the strength of the MHC binding of the peptide(s) involved in sarcoidosis pathogenesis. Our observation that HLA-DRB1*01 and -DRB1*04 phenotype frequencies are reduced in sarcoidosis cases in the majority of populations tested suggests, for the first time, that a common protective mechanism exists. Whether the apparent protective effect of HLA-DRB1 alleles with a hydrophobic position 11 residue is due to altered binding of a foreign antigen or an autoantigen is unclear, as the disease causing antigenic stimulus remains undetermined.

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