Human Leukocyte Antigens A and B in Turkish Patients With Sarcoidosis

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OBJECTIVE: Associations between human leukocyte antigens (HLA) and sarcoidosis have been reported in several studies. We aimed to investigate these associations in Turkish patients.

PATIENTS AND METHOD: We performed HLA-A, HLA-B, HLA-C, and HLA-D typing in 83 patients with sarcoidosis and in 250 healthy controls using a microlymphocytotoxicity method to investigate genetic susceptibility to the disease.

RESULTS: Because of significant violation of Hardy-Weinberg equilibrium at HLA-C and HLA-DQB1 loci, only results obtained at other HLA loci were used. Although HLA-A9, HLA-B5, and HLA-B8 allele frequencies were significantly higher in the patient group compared to the controls (odds ratio [OR]= 21.8, *P*=.015; OR=9.34, *P*=.049; OR=2.26, *P*=.031, respectively), none of the differences remained significant after applying the Bonferroni correction. HLA-A24, HLA-A26, and HLA-B62 alleles were significantly less frequent in the patient group compared to the controls (OR=0.48, *P*=.018; OR=0.19, *P*=.003; OR= 0.11, *P*=.044, respectively). However, the differences also failed to remain significant after Bonferroni correction.

CONCLUSIONS: These results suggest that both HLA may play significant roles (either increasing or reducing risk) in the pathogenesis of sarcoidosis and in its distinct clinical forms and laboratory findings.

Key words: *Human leukocyte antigens. Sarcoidosis. Turkish patients.*

Antígenos leucocitarios humanos A y B en pacientes turcos con sarcoidosis

OBJETIVO: En varios estudios se ha demostrado la existencia de asociaciones entre los antígenos leucocitarios humanos (HLA) y la sarcoidosis. El objetivo de nuestro estudio ha sido la investigación de estas asociaciones en pacientes turcos.

PACIENTES Y MÉTODO: Se ha realizado la tipificación HLA-A, HLA-B, HLA-C y HLA-C en 83 pacientes con sarcoidosis y en 250 controles sanos mediante un método de microlinfocitotoxicidad, con objeto de determinar la susceptibilidad frente a la enfermedad.

RESULTADOS: Debido a la importante violación del equilibrio de Hardy-Weinberg en los *loci* HLA-C y HLA-DQB1, sólo se utilizaron los resultados obtenidos en los demás *loci* HLA. Aunque las frecuencias de los alelos HLA-A9, HLA-B5 y HLA-B8 fueron significativamente mayores en el grupo de pacientes que en el grupo control (cociente de posibilidades [CP] = 21,8, p = 0,015; CP = 9,34, p = 0,049; CP = 2,26, p = 0,031, respectivamente), ninguna de estas diferencias mantuvo la significación estadística tras la aplicación de la corrección de Bonferroni. Los alelos HLA-A24, HLA-A26 y HLA-B62 fueron significativamente menos frecuentes en el grupo de pacientes que en el grupo de controles (CP = 0,48, p = 0,018; CP = 0,19, p = 0,003; CP = 0,11, p = 0,044, respectivamente). Sin embargo, las diferencias tampoco fueron estadísticamente significativas después de la corrección de Bonferroni.

CONCLUSIONES: Estos resultados indican que los HLA pueden desempeñar una función significativa (con aumento o reducción del riesgo) en la patogenia de la sarcoidosis, así como en sus diferentes formas clínicas y en sus alteraciones analíticas.

Palabras clave: *Antígenos HLA. Sarcoidosis. Pacientes turcos.*

Introduction

Sarcoidosis is a systemic disease of unknown etiology characterized histopathologically by the observation of noncaseating granulomas and several immunological abnormalities.[1,2] Although the etiology of the disease remains unclear, infectious and environmental factors based on immunogenetic factors have been postulated. One of the relevant genetic factors has been determined through analysis of major histocompatibility complex genes, especially human leukocyte antigens (HLA).[3] HLA frequencies in patients with sarcoidosis have been studied by many researchers with the aim of discovering the immunogenetic mechanisms in the pathogenesis of the disease. Varying HLA associations have also been reported in sarcoid patients in different ethnic groups.[4] Because the

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pathophysiology of the disease probably involves antigen recognition, processing, and presentation, investigators have looked at various associations with various HLA-related genes.[5] Sarcoidosis has been shown to be associated with HLA-DR3, -DR5, and -DR6 in Caucasian populations,[6-9] and a number of studies of sarcoid subjects belonging to different Caucasian and non-Caucasian ethnic groups have found associations with HLA-B8, -B13, -B5, -B7, and -B35, and HLA-A9.[10] The aim of the present study was to investigate the HLA associations in Turkish patients with sarcoidosis.

Patients and Methods

Patients and Controls

Eighty-three patients with sarcoidosis were studied. The diagnosis was confirmed by lung histopathology with or without the study of other organ biopsies, and all patients had clinical features typical of the disease. Two hundred fifty healthy subjects with no history of sarcoidosis or other lung disease were included as controls. Patients and controls were from the same ethnic group and were not relatives.

The patient group consisted of 21 males and 62 females. The mean (SD) age of the patients at the time of diagnosis was 41.4(13.8) years (range, 14-70 years). At the time of diagnosis, the distribution of stages of disease indicated by chest radiographs were as follows: 1 patient at stage 0 (normal radiograph), 29 patients at stage 1 (bilateral hilar lymphadenopathy), 45 patients at stage 2 (bilateral hilar lymphadenopathy with parenchymal infiltration), 7 patients at stage 3 (parenchymal infiltration without hilar lymphadenopathy), and 1 patient at stage 4 (advanced fibrosis and bullae, cysts). Extrapulmonary sarcoid involvement was detected in 33 (39.8%) of the patients. Five tuberculin units of purified protein derivative were applied using the Mantoux test procedure. The result of a tuberculin skin test was considered positive if an induration was greater than 15 mm in patients who had been vaccinated against tuberculosis and if greater than 10 mm for unvaccinated patients.[11] Positive tuberculin test results were thus recorded for 14 (16.9%) of the patients.

HLA Typing

HLA typing of peripheral blood samples from the patients and controls was carried out using a standard microlymphocytotoxicity assay as previously described.[12] HLA typing was performed using the Biologische Analysensystem Histo Tray ABC 72 and Histo Tray DR 72 (BAG GmbH, Lich, Germany).

Statistical Analysis

The results were evaluated by χ^2 or Fisher exact tests. Where appropriate, a Bonferroni correction was applied to control for the number of comparisons. Odds ratios and 95% confidence intervals (CI) were calculated to assess patients' increased risk of developing sarcoidosis in comparison with controls.[13] To assess the agreement between genotypes observed and those predicted by the Hardy-Weinberg equilibrium, the likelihood ratio (G statistic) was used.[14,15] The linkage disequilibrium parameter (Δ) was calculated to test whether frequencies of alleles from two different loci were independent of each other. *P* values less than .05 were considered to indicate statistical significance. Analyses were carried out with SPSS (version 11.5) and POPGENE (version 1.32) software.

Results

The frequency distributions at HLA-A and HLA-B loci showed that the population sample was in Hardy-Weinberg equilibrium at each locus in the control group (HLA-A: G^2=179.3, degree of freedom=190, *P*=.70; HLA-B: G^2=270.8, degree of freedom=325, *P*=.99). Observed homozygosity showed no significant fluctuations compared with the expected values at HLA-A and HLA-B. Because of significant violation of Hardy-Weinberg equilibrium at HLA-C and HLA-DQB1 loci, and relative unreliability of serologic typing at these loci, only results obtained at HLA-A and HLA-B loci were used.

A total of 22 HLA-A and 34 HLA-B alleles were found. At HLA-A, the most frequent alleles were HLA-A2, -A24, and -A1 for the control group (41.2%, 31.6%, and 21.2%, respectively), and at HLA-A2, -A3, and -A1 for the patient group (41.0%, 24.1%, and 21.7%, respectively). At HLA-B, the most frequent alleles were HLA-B35, -B51, and -B44 for both the control group (39.2%, 27.2%, and 12.4%, respectively) and the patient group (30.1%, 20.5%, and 20.5%, respectively).

HLA-A9 and HLA-B5 and -B8 frequencies were significantly higher in the patients than in the controls. After Bonferroni correction, none of the differences remained statistically significant, however. On the other hand, HLA-A24 and -A26 and HLA-B62 frequencies were significantly lower in the patients compared to the controls, although no statistically significant differences persisted after Bonferroni correction (Table 1).

The frequency of HLA-A26 was significantly higher in patients with positive tuberculin skin tests (n=3, 21.4%; for negative skin tests, n=0, 0%; *P*=.0004).

None of the differences in HLA allele expression reached statistical significance in the comparisons between patients with only pulmonary involvement as opposed to those with both pulmonary and nonpulmonary involvement.

TABLE 1
Significant Human Leukocyte Antigen Frequencies in Turkish Sarcoidosis Patients and Healthy Controls*

HLA	Patients (n=83)	Controls (n=250)	OR (95% CI)	*P*
A9	3 (3.6%)	0 (0%)	21.8 (1.1-426.2)	.015
A24	15 (18.1%)	79 (31.6%)	0.48 (0.26-0.89)	.018
A26	3 (3.6%)	41 (16.4%)	0.19 (0.06-0.64)	.003
B5	3 (3.6%)	1 (0.4%)	9.34 (1.00-91.0)	.049
B8	13 (15.7%)	19 (7.6%)	2.26 (1.1-4.8)	.031
B62	0 (0%)	13 (5.2%)	0.11 (0.01-1.8)	.044

*HLA indicates human leukocyte antigen; OR, odds ratio; CI, confidence interval.

TABLE 2
Human Leukocyte Antigen Linkage Disequilibrium in Patients at *P*<.01

Patients	Δ	χ^2	*P*
A26-B37	0.0069	7.21	.0072
A32-B41	0.0071	9.65	.0019

TABLE 3
Human Leukocyte Antigen Linkage Disequilibrium in Controls at *P*<.01

Controls	Δ	χ^2	*P*
A26-B38	0.0102	7.89	.0050
A33-B17	0.0011	11.20	.0008
A66-B5	0.0011	11.20	.0008
A69-B37	0.0011	6.91	.0086

Statistically significant pairwise ligament disequilibrium was observed in patients at 2 pairs of HLA loci, as shown in Table 2. In contrast, significant pairwise ligament disequilibrium was observed in controls for the 4 pairs of HLA loci shown in Table 3.

Discussion

Sarcoidosis is believed to be triggered by an intricate combination of environmental and genetic factors. Over the last 2 decades many reports of HLA expression in different sarcoid populations have been published in an effort to further our understanding of the immunological and genetic features of the disease.[10,16-27] Although a clear association between HLA typing and sarcoidosis is still disputed, there is nonetheless general agreement that some HLA haplotypes are related to phenotypic variations of the disease.[16]

HLA-A2, -A24, and -A1 and HLA-B35, -B51, and -B44 were the most frequent alleles at the HLA-A and HLA-B loci in our control group. These results are consistent with those found by Uyar et al[28] in a study of a larger population sample.

Previous work on the relationship of sarcoidosis to HLA have produced different results, however. The most common sarcoidosis association has been found to be with the HLA-B8 allele in Caucasians[9,17-19]

Few studies have been published on HLA expression in Turkish sarcoid patients. Akokan et al[29] reported that frequencies of HLA-A9 and HLA-B5 were significantly higher in Turkish patients than in controls, but the number of patients in that study was very small and a Bonferroni correction was not applied to control for the number of comparisons. Consistent with the findings of Akokan et al, we found increased frequency of HLA-A9 and HLA-B5 expression. We also observed a positive HLA-B8 association and negative HLA-A24 and -A26 and HLA-B62 associations in patients. Thus far, no clear conclusion has been reached on HLA haplotypes in Turkish sarcoidosis patients. Although various ethnic groups are present in the Turkish population generally, they have been reported to share a common ancestry, based on similar HLA profiles.[30]

In the German population, an association between sarcoidosis and HLA-DR5 has been reported,[8] and a higher frequency of HLA-DRw52 has been seen in Japanese patients.[4,19] Higher frequencies of HLA-A1, HLA-B7 and -Bw46, HLA-Cw6 and -Cx46, and HLA-DRw8 and -DRw9 have also been demonstrated in sarcoid patients in Japan.[19,27] In West Indian sarcoid patients, a higher frequency of HLA-DR7 has been found, whereas in English patients with sarcoidosis a higher frequency has been identified for HLA-Cw7.[30] An association between HLA-B7 and sarcoidosis in Scandinavia has been reported.[20] An HLA Class I association between HLA-B22 and sarcoidosis in India has been observed, although the association was not statistically significant when the Bonferroni correction was applied.[25]

It has been suggested that HLA-B8 is associated with sarcoidosis.[31] In European Caucasians, HLA-B8 shows linkage disequilibrium with HLA-Cw7.[15] In our study such disequilibrium was found in patients for 2 pairs: for HLA-A26 and HLA-B37 and for HLA-A32 and HLA-B41.

Persson et al[32] reported that the frequency of HLA-B7 was higher in sarcoid patients with positive tuberculin skin tests. However, in our study, it was HLA-A26 that was found to be expressed more frequently in patients with positive skin tests than in patients with negative tests.

Associations between extrapulmonary involvement in sarcoidosis and HLA have been reported as follows for different populations: both HLA-B8 and HLA-A1 with arthritis and erythema nodosum,[6,17,33,34] both HLA-B8 and HLA-DR3 with arthritis,[17,22,23,34] and HLA-A1 with uveitis.[17] In our study, statistical analysis could not be performed for the involvement of each nonpulmonary location because of the small numbers of patients with the same patterns of disease expression. When we compared patients with extrapulmonary involvement to those with pulmonary involvement alone, we found no HLA differences to be statistically significant.

In summary, we found positive associations between sarcoidosis in Turkish Patients and HLA-A9 and HLA-B5 and-B8 expression and negative association for HLA-A24, -A26, and -A62. However, these associations were not statistically significant after Bonferroni correction.

These results suggest the need for new studies with larger patient samples to clarify the HLA profile for sarcoid patients with pulmonary and/or extrapulmonary organ involvement in the Turkish population.

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