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The relationship between the human leukocyte antigen system and Crimean-Congo hemorrhagic fever in the Turkish population☆

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SUMMARY

Objectives: The human leukocyte antigen (HLA) molecules have been shown to be important genetic factors in several diseases. In this study we aimed to evaluate the possible role of HLA genes in the course of Crimean-Congo hemorrhagic fever (CCHF) infection.
Methods: A total of 57 adult patients with CCHF and 43 healthy controls living in the same regions as the patients were included in the study. Severe cases were defined according to previously reported severity criteria.
Results: The frequency of HLA-A*02 was found to be significantly higher in the patients than in the healthy controls ($p = 0.021$). However, a significantly lower frequency of HLA-B*27 was observed in the patients than in the healthy controls ($p = 0.01$). The relative risk (RR) of HLA-A*02 allele for CCHF was found to be 1.93 (95% confidence interval $1.11 < RR < 3.36$). With regard to severe and non-severe cases, there was a significantly greater frequency of HLA-A*23 in severe cases ($p = 0.014$).
Conclusions: The results of this study indicate that while some HLA alleles could constitute a risk factor for acquiring CCHF infection, others could have a protective role against the disease. This study also presents the impact of genetic risk factors on the clinical course of the disease.

1. Introduction

Environmental and host genetic factors affect host susceptibility to infections and other human diseases. The diversity of genes involved in the immune response results in different immunological reactions to infectious pathogens. Many genetic loci lead to human disease susceptibility.[1] A current area of interest in the investigation of infectious diseases is genetic markers. The HLA system, also known as major histocompatibility complex (MHC) in humans, has been shown to be an important genetic factor in several diseases, including infectious diseases, autoimmune diseases, and cancer.[1–3] This gene group is located on the short arm of the sixth chromosome and exhibits extensive polymorphism. The HLA genes are divided into two functionally different classes. Class I antigens (HLA-A, HLA-B, and HLA-C loci) are expressed on somatic cells, and class II antigens (HLA-DR, HLA-DP, and HLA-DQ loci) are expressed on immune cells, i.e. monocytes, macrophages, dendritic cells, and T and B lymphocytes. The HLA loci play a key role in the immune response to microorganisms and susceptibility to several infectious diseases.[1,2]

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne viral hemorrhagic disease with a mortality rate of 5–30%.[4,5] The clinical spectrum of this disease varies from a subclinical infection to severe disease and death.[6] No effective antiviral therapy is yet available.[7,8] This disease is an endemic zoonosis in Turkey, appearing during the spring and summer seasons every year.[4] Endemic areas are located in the Black Sea region, and the dominant strains are the serotypes related to the strains from southern Russia and the Balkan countries.[4,9,10]

The pathogenesis of CCHF is a complex process and has not been described completely. Although endothelial damage and an impaired immunological response to the virus are well defined, the relationship between host genetics and susceptibility to the infection is not yet known.[11,12] In this study we aimed to evaluate the possible role of HLA genes in the susceptibility of the host to CCHF infection and to investigate the effect of these molecules on the severity of the disease.

2. Materials and methods

2.1. Patients and control group

This prospective case-controlled study was carried out during the 2009–2010 seasons. A total of 57 adult patients hospitalized with a diagnosis of CCHF were included in the study. The diagnosis

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was confirmed by detection of positive IgM antibody and/or viral genome in patient serum samples. Molecular and serological analyses were performed at the Virology Reference Laboratory of the Refik Saydam National Public Health Agency (Ankara, Turkey). The TaqMan-based one-step reverse-transcriptase PCR assay (RT-PCR) was used to detect CCHF viral RNA.[13] The presence of CCHF-specific serum IgM antibodies was determined by in-house ELISA method. The antigens used in the ELISA tests were obtained from the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA). Patient demographic, clinical, and laboratory data were recorded on individual forms. The control group ($n = 43$) was chosen from healthy solid organ donors who presented to the Organ Transplantation Center and who were living in the same regions as the CCHF patients. Ethics committee approval for genetic studies was obtained. All patients and control group members provided informed consent for participation in a genetic study.

2.2. HLA typing

HLA typing for A, B, Cw, DQB, and DRB loci was done using a medium-resolution PCR sequence-specific oligonucleotide (PCR-SSO) technique. For this purpose, LIFECODES HLA SSO typing kits were provided by Gen-Probe Transplant Diagnostics (Gen-Probe Transplant Diagnostics, Inc., Stamford, CT, USA). When the typing results indicated more than two possible alleles, high-resolution PCR kits with sequence-specific primers (PCR-SSP; Olerup SSP AB, Saltsjöbaden, Sweden) were used to resolve ambiguities. The frequencies of HLA alleles, haplotypes, and genotypes were determined for all of the patients and control group.

2.3. Definition of severe cases

Previously reported disease severity criteria were used to define the severe cases.[14] Patients with at least one of the following were considered as severe cases: somnolence, melena, activated partial thromboplastin time (APTT) $\geq$60 s, and thrombocyte count $\leq 20 \times 10^9$/l. Fatal cases were also included in the severe group.

2.4. Statistical analysis

The frequencies of HLA alleles were compared between the patients and control group and between the non-severe and severe cases. Statistical analyses were performed using SPSS 15.0 statistical package (SPSS Inc., Chicago, IL, USA). Variables were compared by Chi-square test and Fisher's exact test with Yates' correction. The level of significance was set at a p-value of <0.05.

3. Results

The study was performed on 57 adult CCHF patients (31 male, 26 female) and 43 healthy controls (22 male, 21 female). There was no significant difference in the area of residence between the patients and controls ($p = 0.283$). In the patient group, HLA-A*02, HLA-B*35, HLA-Cw*04, HLA-DRB1*11, and HLA-DQB1*03 had the highest frequencies in their own HLA loci (Table 1). When comparing the frequencies of HLA alleles between the patients and the control group, the frequency of HLA-A*02 was found to be significantly higher in the patients than in the healthy controls ($p = 0.021$). On the other hand, a significantly lower frequency of HLA-B*27 was observed in the patients than in the healthy controls ($p = 0.01$).

According to the categorization of the patients by severity criteria, 20 patients were assigned to the severe group and 37 patients were assigned to the non-severe group. Eleven patients in the severe group had a fatal outcome. In the severe and non-severe

Table 1
The frequencies of HLA-A, HLA-B, HLA-Cw, HLA-DQB1, and HLA-DRB1 alleles in patients and controls

HLA allele	CCHF patients, n (%)	Control group, n (%)	p-Value
A*01	16 (14.0)	9 (10.5)	NS
A*02	36 (31.6)	14 (16.3)	0.013[a]
A*03	13 (11.4)	12 (14.0)	NS
A*11	8 (7.0)	12 (14.0)	NS
A*23	4 (3.5)	5 (5.8)	NS
A*24	17 (14.9)	15 (17.4)	NS
A*26	6 (5.3)	5 (5.8)	NS
A*29	-	4 (4.7)	-
A*30	2 (1.8)	2 (2.3)	NS
A*31	4 (3.5)	-	-
A*32	6 (5.3)	4 (4.7)	NS
A*33	1 (0.9)	-	-
A*66	-	1 (1.2)	-
A*68	1 (0.9)	2 (2.3)	NS
A*69	-	1 (1.2)	-
B*04	-	1 (1.2)	-
B*07	10 (8.8)	4 (4.7)	NS
B*08	2 (1.8)	4 (4.7)	NS
B*13	3 (2.6)	2 (2.3)	NS
B*14	1 (0.9)	-	-
B*15	5 (4.4)	2 (2.3)	NS
B*18	5 (4.4)	6 (7.0)	NS
B*27	2 (1.8)	9 (10.5)	0.007[a]
B*35	18 (15.8)	14 (16.3)	NS
B*37	2 (1.8)	3 (3.5)	NS
B*38	5 (4.4)	5 (5.8)	NS
B*39	1 (0.9)	-	-
B*40	6 (5.3)	3 (3.5)	NS
B*41	4 (3.5)	3 (3.5)	NS
B*44	12 (10.5)	7 (8.1)	NS
B*48	2 (1.8)	1 (1.2)	NS
B*49	5 (4.4)	4 (4.7)	NS
B*50	2 (1.8)	-	-
B*51	17 (14.9)	11 (12.8)	NS
B*52	4 (3.5)	1 (1.2)	NS
B*53	1 (0.9)	-	-
B*55	4 (3.5)	1 (1.2)	NS
B*56	-	1 (1.2)	-
B*57	2 (1.8)	2 (2.3)	NS
B*58	-	1 (1.2)	-
B*60	-	1 (1.2)	-
B*78	1 (0.9)	-	-
Cw*01	4 (3.5)	3 (3.5)	NS
Cw*02	7 (6.1)	8 (9.3)	NS
Cw*03	8 (7.0)	6 (7.0)	NS
Cw*04	19 (16.7)	21 (24.4)	NS
Cw*05	3 (2.6)	3 (3.5)	NS
Cw*06	11 (9.6)	6 (7.0)	NS
Cw*07	19 (16.7)	15 (17.4)	NS
Cw*08	3 (2.6)	1 (1.2)	NS
Cw*12	15 (13.2)	9 (10.5)	NS
Cw*14	9 (7.9)	3 (3.5)	NS
Cw*15	8 (7.0)	7 (8.1)	NS
Cw*16	6 (5.3)	3 (3.5)	NS
Cw*17	2 (1.8)	1 (1.2)	NS
DRB1*01	3 (2.6)	-	-
DRB1*03	9 (7.9)	6 (7.0)	NS
DRB1*04	21 (18.4)	18 (20.9)	NS
DRB1*07	8 (7.0)	11 (12.8)	NS
DRB1*08	1 (0.9)	2 (2.3)	NS
DRB1*09	3 (2.6)	1 (1.2)	NS
DRB1*10	1 (0.9)	-	-
DRB1*11	25 (21.9)	25 (29.1)	NS
DRB1*12	2 (1.8)	1 (1.2)	NS
DRB1*13	16 (14.0)	7 (8.1)	NS
DRB1*14	2 (1.8)	3 (3.5)	NS
DRB1*15	16 (14.0)	9 (10.5)	NS
DRB1*16	7 (6.1)	3 (3.5)	NS
DQB1*02	16 (14)	15 (17.4)	NS
DQB1*03	52 (45.6)	45 (52.3)	NS
DQB1*04	1 (0.9)	3 (3.5)	NS
DQB1*05	16 (14.0)	12 (14.0)	NS
DQB1*06	29 (25.4)	11 (12.8)	NS

NS, not significant. Note: the sums of all alleles in CCHF patients and in the control group were 114 and 86, respectively.

[a] Level of significance <0.05 reached in comparisons: OR 0.421, 95% CI 0.210–0.845; RR 1.93, 95% CI $1.11 < RR < 3.36$, for HLA-A*02; OR 6.545, 95% CI 1.376–31.130, for HLA-B*27.