CLINICAL AND VACCINE IMMUNOLOGY, Sept. 2011, p. 1435–1440 Vol. 18, No. 9
1556-6811/11/$12.00 doi:10.1128/CVI.05187-11

HLA-Associated Hemorrhagic Fever with Renal Syndrome Disease Progression in Slovenian Patients[▽]

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Received 23 May 2011/Returned for modification 27 June 2011/Accepted 13 July 2011

Major histocompatibility complex (MHC) class I and class II genes regulate the balance between appropriate aggressive responses and invading pathogens while minimizing the destruction of host tissue. Several studies have shown that in hemorrhagic fever with renal syndrome (HFRS) patients, the disease outcome is determined by a complex interaction between the virus and immunopathologic and human genetic factors. In Slovenia, the severity of the disease caused by Puumala virus (PUUV) is significantly lower than that of HFRS due to Dobrava virus (DOBV). We have determined 23 different HLA-B and 12 different HLA-DRB1 types in Slovenian HFRS patients. Comparison of HLA frequencies between healthy individuals and HFRS patients showed no strong association with the susceptibility for hantaviral infection. Significant associations were recognized when the patient group was separated according to the virus responsible for the infection. DOBV-infected patients have a significantly higher frequency of HLA-B*35 than PUUV-infected patients. For HLA class II genes, the biggest difference between the PUUV- and DOBV-infected groups of patients was in HLA-DRB1*13, where this phenotype was more frequent in PUUV-infected patients, especially in the severe form of the disease. HLA-B*07 could play a protective role in PUUV-caused HFRS in the Slovenian population. Our study shows diverse associations of HLA molecules with DOBV- and PUUV-induced HFRS, and therefore, we presume that different hantaviruses are presented differently through the same HLA molecules and that this might lead to either a more severe or a milder form of the disease. In line with this idea, we have noticed that HLA-B*35 might be a genetic risk factor for DOBV infection in the Slovenian population.

The genus *Hantavirus*, family *Bunyaviridae*, encompasses several pathogenic viruses which cause two systemic infectious diseases, hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. In Europe, hantavirus disease is an endemic zoonosis that affects tens of thousands of individuals per year (11). Puumala virus (PUUV), Dobrava virus (DOBV), Saaremaa virus (SAAV), Tula virus (TULV), and Seoul virus (SEOV) have been proven to circulate in Europe, but the disease is mainly caused by PUUV in northern and western Europe and by DOBV in the central Europe and Balkan region (11).

Slovenia is a country in central Europe touching the Alps and bordering the Mediterranean. It covers an area of 20,273 square kilometers and has a population of 2.06 million people. Despite being a small country, it was shown that four types of hantaviruses, but not SEOV, circulate in the country (1, 2, 17).

In Slovenia, HFRS is caused by PUUV and DOBV hantaviruses, but the clinical severity varies greatly. In all, PUUV is usually the cause of a milder form of disease and DOBV is mainly responsible for more severe cases of the disease, with up to a 10% mortality rate (3, 25). The disease is clinically characterized by a sudden onset of acute fever, headache, back and abdominal pain, myalgia, nausea, vomiting, and transient myopia. In more severe cases, acute renal insufficiency needs to be treated with transient hemodialysis, and hemorrhagic manifestations due to capillary leak syndrome are observed (8, 20).

Hantavirus pathogenesis is likely to be a complex multifactorial process that includes contributions from immune responses, platelet dysfunction, and the deregulation of endothelial cell barrier functions (21). In humans, hantaviruses elicit a strong immune response which seems to be an essential part of virus-associated pathogenesis (27). A mixed pattern of Th1 and Th2 immune response patterns and high levels of proinflammatory cytokines and their insufficient suppression by regulatory cytokines lead to harmful effects of the immune response in HFRS-infected patients (29).

The root of the adaptive immune response in humans involves the human leukocyte antigen (HLA) system. The HLA genes involved in the immune response fall into two functionally different classes: class I genes are expressed in most somatic cells, but the level of expression varies depending on the tissue involved. Class II genes are expressed only on B cells, activated T cells, macrophages, dendritic cells, and thymic epithelial cells. The role of both HLA classes is the presentation of short, pathogen-derived peptides to T cells and the consequent initiation of the adaptive immune response (15, 16). Besides the fact that the class I molecules are essential to the acquired immune response, they are also important in innate immunity as ligands for the killer cell immunoglobulin-like receptors (KIR) on the surface of natural killer cells. In contrast to the case for many bacterial and parasitic infections, an effective host response against viruses that contravene early innate defenses relies heavily on HLA-restricted T-cell responses (5). A genetic predisposition toward severe HFRS

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▽ Published ahead of print on 20 July 2011.

disease caused by PUUV infection was shown to be associated especially with the haplotype HLA-B*08 DRB1*03:01 (22, 24, 26). Individuals with the haplotype HLA-B*08 DRB1*03:01 are prone to higher antibody production and lower production of regulatory cytokines (6). The same HLA haplotype was linked to a more rapid progression from HIV infection to AIDS (13) and with failure of the response against hepatitis B vaccine (9). In contrast, the HLA B*27 molecule, a known marker for autoimmune diseases (15), was associated with a more benign clinical course of HFRS disease in patients with PUUV infection (24) and significantly improved survival in HIV-infected individuals (7). For hantavirus infections, an association between HLA molecules and the clinical course of the disease was also shown for Sin Nombre virus (SNV), where patients with B*35:01 or DRB1*14:02 were more likely to experience a severe course of the disease or even a fatal outcome (14). In patients infected with Andes virus (ANDV), HLA B*08 was again correlated with the severe course of the disease, but HLA DRB1*15 alleles were significantly more common in a group of patients with the mild course of the disease (10).

The immunogenetic relationships between severe forms of hantaviral disease in humans are complex, but several parallels between the same alleles and different viruses suggest further investigation. In this study, we have investigated a correlation between HLA type and disease progression in hospitalized patients with HFRS in Slovenia. This study represents the first determination of HLA molecules and analysis of their possible impact on the clinical course of the disease in patients infected with DOBV.

MATERIALS AND METHODS

Patients and control group. A total of 160 patients hospitalized in Slovenia with the diagnosis of hemorrhagic fever with renal syndrome were included in the study. Of these, 72 patients were diagnosed with DOBV infection and 88 patients with PUUV infection. During the course of the disease, the clinical diagnosis was confirmed serologically by an indirect immunofluorescence assay and by enzyme-linked immunoassay IgM and IgG tests or with one-step quantitative reverse transcription (RT)-PCR assay, as described previously (3, 18, 28).

A control group consisted of 131 randomly chosen healthy unrelated individuals. All subjects were of Slovenian nationality.

Clinical data collection. For all patients included in the study, the clinical data were collected retrospectively. In addition, patients were categorized into three different groups based on the severity of the disease. The severity of the disease was graded on the basis of clinical and laboratory parameters used in the proposed Croatian scale for grading disease severity in patients with HFRS (19), which has previously been evaluated on a group of Slovenian HRFS patients (28).

The first group comprised the patients with a fatal outcome of the disease. The second group consisted of patients with severe disease, and the third group of patients had a mild disease progression. In more detail, patients with severe disease were classified as having increased serum creatinine and urea levels (maximum values of at least 4 times the normal value), the need for hemodialysis, and the presence of hemorrhagic manifestations. Since the study was retrospective, informed consent was not obtained from patients. The research protocol was approved by the National Medical Ethics Committee of the Republic of Slovenia.

HLA typing. Genomic DNA was extracted from whole-blood samples using a DNA minikit (Qiagen) or with a DNA blood kit on an EZ1 BioRobot (Qiagen), depending on the available sample volume.

Individuals with HFRS and the control group were typed for HLA-B and HLA-DRB1 with LABType SSO typing tests (LT SSO B Locus and LT SSO DRB1 by One Lambda, Canoga Park, CA) using Luminex technology on a flow cytometer-like instrument (Labscan 100, Luminex Corporation, Austin, TX). This product is a reverse sequence-specific-oligonucleotide DNA typing assay that uses sequence-specific oligonucleotide probes and color-coded microspheres to identify the HLA allele group.

Statistical analysis. A computer program was developed for statistical analysis. The two-tailed Fischer's exact test was used for comparison of the frequencies of individual alleles between the control group and HFRS patients. Among HFRS patients, comparisons were made between those infected with PUUV and with DOBV and according to the disease progression. The level of significance was set at a P value of <0.05.

RESULTS

In total, the study was performed on 88 HFRS patients hospitalized due to PUUV infection and 72 HFRS patient with DOBV infection. On the basis of their clinical and laboratory parameters during the hospitalization period, patients infected with PUUV were categorized into two groups: 51 patients were assigned to the mild group and 37 patients to the severe group. In addition, 72 patients hospitalized due to DOBV infection were sorted into three groups consisting of 7 patients with a fatal outcome, 31 patients in the severe group, and 29 patients in the mild group.

Comparison of HLA-B and HLA-DRB1 phenotype frequencies in a control group versus their frequencies in patients with HFRS due to either DOBV or PUUV infection. Altogether, we determined 23 different HLA-B and 12 different DRB1 phenotypes in Slovenian HFRS patients (Table 1). Additionally, we examined 131 healthy individuals who represent a control group in the study. The frequencies established in the control group correspond to HLA frequencies in the Slovenian general population (Table 1). From HLA class I loci, only allele group HLA-B*53 showed a significant difference ($P = 0.040$) between its frequency in HFRS patients and in the control group, but this HLA type is very low in frequency in Slovenian population and was not present at all among HFRS patients. Besides HLA class I molecules, a significantly lower frequency of DRB1*03 ($P = 0.049$) was also observed in the patient group than in the control group. The lower frequency of this type was also observed when the group of patients was divided according to the virus responsible for the infection. In the group of PUUV-infected patients, the frequency of HLA-DRB1*03 was lower than its frequency in the control group (12.5% versus 23.7%; $P = 0.053$). When comparing DOBV-infected patients and the control group, no significant differences in frequencies were observed for HLA class I or II. The only distinction was a greater frequency of HLA-B*35 among patient groups infected with DOBV (34.3% versus 23.7%).

When comparing allele group frequencies of HLA-B between patients infected with DOBV and PUUV, a statistical difference was observed in the frequencies of HLA-B*35 ($P = 0.027$). The frequency of HLA-B*35 was significantly higher among DOBV-infected patients than in PUUV-infected patients (34.3% versus 18.4%). A borderline significance ($P = 0.068$) was also noticed for HLA-DRB1*13, which was, in contrast, more common in the PUUV-infected group of patients (18.3% versus 31.8%).

Comparison of HLA typing in PUUV-infected HFRS patients with different disease progressions. The results for comparison of HLA typing in PUUV-infected HFRS patients with the different disease progressions are presented in Table 2. The potential effects of HLA-B and HLA-DRB1 on disease progression were evaluated in 88 PUUV-infected Slovenian

TABLE 1. Frequencies of HLA-B and HLA-DRB1 were determined for HFRS patients and the control group and compared among groups of patients and/or the control group[a]

Allele	Frequency (%) among individuals in indicated group				*P* value (relative risk)[b]			
	Control (n = 131)	HFRS (n = 160)	DOBV (n = 72)	PUUV (n = 88)	HFRS/control	DOBV/control	PUUV/control	DOBV/PUUV
B*07	24.4	18.1	19.4	18.4	NS	NS	NS	NS
B*08	13.7	11.3	11.9	11.5	NS	NS	NS	NS
B*13	6.9	8.1	6.0	10.3	NS	NS	NS	NS
B*14	3.1	5.6	7.5	4.6	NS	NS	NS	NS
B*15	7.6	8.8	6.0	11.5	NS	NS	NS	NS
B*18	16.0	12.5	9.0	16.1	NS	NS	NS	NS
B*27	13.0	18.8	19.4	19.5	NS	NS	NS	NS
B*35	23.7	24.4	34.3	18.4	NS	NS	NS	0.027
B*37	3.1	0.6	0.0	1.2	NS	NS	NS	NS
B*38	8.4	8.1	7.5	9.2	NS	NS	NS	NS
B*39	3.1	3.1	4.5	2.3	NS	NS	NS	NS
B*40	9.9	8.1	6.0	10.3	NS	NS	NS	NS
B*41	1.5	1.9	1.5	2.3	NS	NS	NS	NS
B*44	17.6	17.5	23.9	13.8	NS	NS	NS	NS
B*47	0.0	1.3	1.5	1.2	NS	NS	NS	NS
B*49	4.6	3.1	3.0	3.5	NS	NS	NS	NS
B*50	3.1	2.5	4.5	1.2	NS	NS	NS	NS
B*51	13.0	17.5	16.4	19.5	NS	NS	NS	NS
B*52	2.3	1.3	0.0	2.3	NS	NS	NS	NS
B*53	3.1	0.0	0.0	0.0	0.040 (0.0)	NS	NS	NS
B*55	4.6	4.4	4.5	4.6	NS	NS	NS	NS
B*56	0.8	1.9	0.0	3.5	NS	NS	NS	NS
B*57	6.9	6.9	6.0	8.1	NS	NS	NS	NS
B*58	3.1	0.6	0.0	1.2	NS	NS	NS	NS
DRB1*01	28.2	29.4	33.8	26.1	NS	NS	NS	NS
DRB1*03	23.7	14.4	16.9	12.5	0.049 (0.737)	NS	0.053* (0.602)	NS
DRB1*04	16.0	17.5	18.3	17.1	NS	NS	NS	NS
DRB1*07	18.3	22.5	22.5	22.7	NS	NS	NS	NS
DRB1*08	3.1	5.0	5.6	4.6	NS	NS	NS	NS
DRB1*10	0.8	1.3	0.0	2.3	NS	NS	NS	NS
DRB1*11	26.0	25.6	23.9	27.3	NS	NS	NS	NS
DRB1*12	0.8	2.5	4.2	1.1	NS	NS	NS	NS
DRB1*13	20.6	25.6	18.3	31.8	NS	NS	0.080* (1.392)	0.068*
DRB1*14	8.4	8.8	12.7	5.7	NS	NS	NS	NS
DRB1*15	22.1	19.4	16.9	21.6	NS	NS	NS	NS
DRB1*16	16.0	10.6	9.9	11.4	NS	NS	NS	NS

[a] The groups comprised a control group of healthy individuals and a group of HFRS patients which was additionally separated into a DOBV-infected group and a PUUV-infected group.

[b] *P* values between indicated groups were determined using the two-tailed Fischer's exact test. NS, not significant; *, borderline significant ($P < 0.1$).

patients. The HLA-B*08 haplotype was more frequent among our PUUV-infected patients with the severe outcome (18.9% versus 6.0%; $P = 0.090$). Similarly, HLA-B*15 was more frequent in patients with the severe disease progression than in patients with the mild form of the disease (18.9% versus 6.0%; $P = 0.090$). Moreover, HLA-B*56 was noticed significantly more frequently in the severe group of patients than in the control group (8.1% versus 0.8%; $P = 0.034$). On the other hand, there was a significantly lower frequency of HLA-B*07 among patients with the severe form of the disease than in the mild group of patients (8.1% versus 26.0%; $P = 0.049$). Besides the alleles already pointed out, HLA-B*57 was also significantly different when comparing groups of patients with the mild and the severe form of the disease (14.0% versus 0.0%; $P = 0.019$). The frequency of allele group HLA-B*57 was, in contrast, two times higher in the group of patients with the mild disease progression than in the control group (14.0% versus 6.9%), but the difference was not significant. Our results show that the frequency of HLA-DRB1*03 was significantly lower in the group of patients with mild disease progression than in the control group (7.8% versus 23.7%; $P = 0.020$). HLA-DRB1*15 is suggested to have a protective role, since its frequency was significantly lower in the severe group than in the mild group of patients (8.1% versus 31.4%; $P = 0.009$). On the other hand, allele group HLA-DRB1*13 could be associated with the severe progression of the disease, since the frequency of this allele group in the severe group was 43.2% versus 23.5% in the mild group of patients (severe versus mild; $P = 0.065$) and 20.6% in the control group (severe versus control; $P = 0.010$).

Comparison of HLA typing in DOBV-infected HFRS patients with different disease progressions. HFRS patients infected with DOBV were sorted into three groups according to disease progression, and the HLA-B and HLA-DRB1 frequencies were compared among groups of patients and the control group (Table 3). In contrast to the patient group infected with PUUV, no statistically significant differences were noted among DOBV-infected patients with different clinical progressions. However, we observed higher frequencies of HLA-B*35, HLA-B*57, HLA-DRB1*01, and HLA-DRB1*11, especially

TABLE 2. Frequencies of HLA-B and HLA-DRB1 were determined for PUUV-infected patients with different disease progressions and compared among groups

Allele	Frequency (%) among individuals in indicated group			*P* value (relative risk)[a]		
	Control (n = 131)	Outcome		Severe/mild	Mild/control	Severe/control
		Severe (n = 37)	Mild (n = 51)			
B*07	24.4	8.1	26.0	0.049 (0.392)	NS	0.038 (0.335)
B*08	13.7	18.9	6.0	0.090* (1.797)	NS	NS
B*13	6.9	13.5	8.0	NS	NS	NS
B*14	3.1	2.7	6.0	NS	NS	NS
B*15	7.6	18.9	6.0	0.090* (1.703)	NS	0.062* (2.073)
B*18	16.0	13.5	18.0	NS	NS	NS
B*27	13.0	21.6	18.0	NS	NS	NS
B*35	23.7	13.5	22.0	NS	NS	NS
B*37	3.1	0.0	2.0	NS	NS	NS
B*38	8.4	13.5	6.0	NS	NS	NS
B*39	3.1	2.7	2.0	NS	NS	NS
B*40	9.9	5.4	14.0	NS	NS	NS
B*41	1.5	0.0	4.0	NS	NS	NS
B*44	17.6	13.5	14.0	NS	NS	NS
B*47	0.0	2.7	0.0	NS	NS	NS
B*49	4.6	5.4	2.0	NS	NS	NS
B*50	3.1	2.7	0.0	NS	NS	NS
B*51	13.0	18.9	20.0	NS	NS	NS
B*52	2.3	0.0	4.0	NS	NS	NS
B*53	3.1	0.0	0.0	NS	NS	NS
B*55	4.6	5.4	4.0	NS	NS	NS
B*56	0.8	8.1	0.0	0.073* (2.471)	NS	0.034 (3.458)
B*57	6.9	0.0	14.0	0.019 (0.0)	NS	NS
B*58	3.1	2.7	0.0	NS	NS	NS
DRB1*01	28.2	18.9	31.4	NS	NS	NS
DRB1*03	23.7	18.9	7.8	NS	0.020 (0.359)	NS
DRB1*04	16.0	24.3	11.8	NS	NS	NS
DRB1*07	18.3	21.6	23.5	NS	NS	NS
DRB1*08	3.1	2.7	5.9	NS	NS	NS
DRB1*10	0.8	5.4	0.0	NS	NS	NS
DRB1*11	26.0	32.4	23.5	NS	NS	NS
DRB1*12	0.8	2.7	0.0	NS	NS	NS
DRB1*13	20.6	43.2	23.5	0.065* (1.633)	NS	0.010 (2.215)
DRB1*14	8.4	2.7	7.8	NS	NS	NS
DRB1*15	22.1	8.1	31.4	0.009 (0.320)	NS	0.060* (0.375)
DRB1*16	16.0	13.5	9.8	NS	NS	NS

[a] *P* values between control group and/or groups with indicated outcomes were determined using the two-tailed Fischer's exact test. NS, not significant; *, borderline significant ($P < 0.1$).

among DOBV-infected patients with a fatal outcome of the disease. A greater frequency of HLA-B*35 was observed when comparing patients with a fatal outcome of the disease and the control group (57.1% versus 23.7%; $P = 0.069$). Also, the frequency of allele group HLA-B*57 was greater in the group of patients with a fatal outcome than in the mild group of patients ($P = 0.090$) or the severe group of patients ($P = 0.081$). In addition, HLA-B*27 was more frequent among patients with the severe disease progression, and the frequency was twice as high as in the control group ($P = 0.096$).

DISCUSSION

In HFRS patients, the disease outcome is determined by a complex interaction between the virus and immunopathologic and human genetic factors. In Slovenia, DOBV and PUUV coexist in a single region of endemicity, and the disease caused by PUUV is significantly less severe than that of HFRS due to DOBV (3). Beyond that, differences in disease severity among the HFRS cases caused by DOBV have been noticed (3, 4). In our study reported here, we examined HLA-B and HLA-DRB1 molecules in Slovenian HFRS patients.

First, a comparison was made between healthy individuals from the Slovenian population and HFRS patients. No strong association was observed except for HLA-B*53 and HLA-DRB1*03, where decreased frequencies were present in the patient group. In any case, in our opinion, HLA-B and HLA-DRB1 are not significantly associated with the susceptibility of an individual to hantaviral infection. More significant associations were recognized when the patient group was separated according to the virus responsible for the infection. DOBV-infected patients have a significantly higher frequency of HLA-B*35 than PUUV-infected patients, especially those with a severe outcome of the disease. An association between HLA-B*08 DRB1*03 and a severe progression of HFRS caused by PUUV has previously been reported for the Finnish population (22, 23). In our study, HLA-B*08 was also associated with a severe disease progression in PUUV-infected patients, but

TABLE 3. Frequencies of HLA-B and HLA-DRB1 were determined for DOBV-infected patients with different disease progressions and compared among groups

Allele	Frequency (%) among individuals in indicated group				*P* value (relative risk)[a]					
	Control ($n = 131$)	Outcome			Fatal/severe	Fatal/mild	Severe/mild	Fatal/control	Severe/control	Mild/control
		Fatal ($n = 7$)	Severe ($n = 31$)	Mild ($n = 29$)						
B*07	24.4	14.3	29.0	10.3	NS	NS	NS	NS	NS	NS
B*08	13.7		12.9	13.8	NS	NS	NS	NS	NS	NS
B*13	6.9	14.3		10.3	NS	NS	NS	NS	NS	NS
B*14	3.1		6.5	10.3	NS	NS	NS	NS	NS	NS
B*15	7.6	14.3	6.5	3.5	NS	NS	NS	NS	NS	NS
B*18	16.0	14.3	9.7	6.9	NS	NS	NS	NS	NS	NS
B*27	13.0	14.3	25.8	13.8	NS	NS	NS	NS	0.096* (1.906)	NS
B*35	23.7	57.1	32.3	31.0	NS	NS	NS	0.069* (3.924)	NS	NS
B*38	8.4		9.7	6.9	NS	NS	NS	NS	NS	NS
B*39	3.1		3.2	6.9	NS	NS	NS	NS	NS	NS
B*40	9.9		6.5	6.9	NS	NS	NS	NS	NS	NS
B*41	1.5			3.5	NS	NS	NS	NS	NS	NS
B*44	17.6		29.0	24.1	NS	NS	NS	NS	NS	NS
B*47	0.0			3.5	NS	NS	NS	NS	NS	NS
B*49	4.6		3.2	3.5	NS	NS	NS	NS	NS	NS
B*50	3.1		3.2	6.9	NS	NS	NS	NS	NS	NS
B*51	13.0		12.9	24.1	NS	NS	NS	NS	NS	NS
B*55	4.6	14.3	3.2	3.5	NS	NS	NS	NS	NS	NS
B*57	6.9	28.6	3.2	3.5	0.081* (4.667)	0.090* (4.340)	NS	0.098* (4.618)	NS	NS
DRB1*01	28.2	42.9	34.4	31.3	NS	NS	NS	NS	NS	NS
DRB1*03	23.7		18.8	18.8	NS	NS	NS	NS	NS	NS
DRB1*04	16.0		18.8	21.9	NS	NS	NS	NS	NS	NS
DRB1*07	18.3	28.6	21.9	21.9	NS	NS	NS	NS	NS	NS
DRB1*08	3.1		3.1	9.4	NS	NS	NS	NS	NS	NS
DRB1*11	26.0	42.9	25.0	18.8	NS	NS	NS	NS	NS	NS
DRB1*12	0.8		3.1	6.3	NS	NS	NS	NS	NS	NS
DRB1*13	20.6		18.8	21.9	NS	NS	NS	NS	NS	NS
DRB1*14	8.4	14.3	9.4	15.6	NS	NS	NS	NS	NS	NS
DRB1*15	22.1	14.3	18.8	15.6	NS	NS	NS	NS	NS	NS
DRB1*16	16.0	14.3	9.4	9.4	NS	NS	NS	NS	NS	NS

[a] *P* values between control group and/or groups with indicated outcomes were determined using the two-tailed Fischer's exact test. NS, not significant; *, borderline significant ($P < 0.1$).

the difference in allele group frequencies was only borderline significant. No such association was observed among DOBV-infected patients. The only association between the severity of the disease and HLA haplotype in DOBV-caused HFRS was seen in the group of patients with a fatal outcome, where a greater frequency of HLA-B*35 was noted. The same HLA type has already been reported in correlation with a severe form of HCPS induced by SNV (14) and an accelerated progression to AIDS (12). Additionally, a significantly greater frequency of allele group HLA-B*56 was observed in PUUV-infected patients, especially in those with the severe disease progression. This implies that selected alleles could be a risk factor for a more severe form of PUUV-caused HFRS. Significant associations between HLA type and the severe progression of PUUV-caused disease were established for HLA class II DRB1 alleles. The greatest difference between the PUUV- and the DOBV-infected groups of patients was in HLA-DRB1*13, which was more frequent in PUUV-infected patients, especially in those with the severe form of the disease. In addition, HLA-DRB1*03 was more frequent in the severe group of patients infected with PUUV than in the mild group, but the frequency was comparable to that in the control group. In the group of patients with the mild form of the disease, the selected HLA type was significantly less frequent than in the control group. HLA-DRB1 alleles have already been connected to the severe disease progression for PUUV-infected patients in the Finnish population (HLA-DBR1*03) (22, 23) and also with a mild form of ANDV-caused HCPS in the Chilean population (HLA-DRB1*15) (10). Similar results were observed in the Chinese Han population, where HLA-DRB1*13 and HLA-DRB1*15 were detected in fewer individuals with Hantaan virus (HTNV)-induced HFRS than in the control population, but no significant correlation was established between susceptibility to HTNV and selected allele groups (31). In the Slovenian population, a mild form of PUUV-caused HFRS was associated with HLA-DRB1*15; therefore, we think that this type might have a protective role against a severe form of the disease but does not influence the susceptibility to infection. Unfortunately, no such observation was found in DOBV-infected patients, which implies that different hantaviruses are differently processed through HLA molecules. In addition, it has been previously reported that the allele group HLA-B*27 has a protective role in PUUV- and SNV-induced disease (14, 24), but no parallel could be confirmed among Slovenian patients, neither those infected with PUUV nor those infected with DOBV. A protective role of

HLA type in the Slovenian population against the severe form of PUUV-caused HFRS could be associated with HLA-B*07, which was statistically more frequent in HFRS patients with the mild disease progression.

Major histocompatibility complex (MHC) class I and class II genes regulate the balance between appropriate aggressive responses to invading pathogens and minimized destruction of host tissue (30). Different studies concerning genetic susceptibility and the disease progression of HFRS have shown diverse associations of HLA molecules and hantavirus infection (10, 14, 22, 23, 31). Based on that and the results from our study, which show diverse associations of HLA molecules for DOBV- and PUUV-induced HFRS, we presume that different hantaviruses are presented differently through the same HLA molecules and that that might lead to either the more severe or the mild form of the disease. In their study, Wang et al. hypothesized that HLA-B*08 DRB1*3 and B*46 DRB1*09 haplotypes, which are very frequent in the Caucasian and Chinese populations, respectively, are two genetic risk factors for HFRS in those populations (31). In line with that observation, we have noticed that HLA-B*35 might be a genetic risk factor for the same disease in the Slovenian population. HLA-B*35 is a common HLA type in our population, so it is possible that hantaviruses adapt to the most frequent allele groups in a selected population. Although genetic epidemiological studies of HLA are important, they still require complementation by other, mostly large-scale studies to confirm their results because, as in the case of most human-pathogen interactions, the hantavirus disease outcome cannot be exclusively explained by human genetic factors. Our study has confirmed some previous findings about HLA type influencing the hantavirus disease outcome and exposed some new possible associations. In order to confirm our observations, further studies are needed in the Slovenian population, as well as in other countries with hantavirus infections.

ACKNOWLEDGMENT

The authors state that no potential conflict of interest exists.

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