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Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus

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Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. Nephropathia epidemica (NE) caused by Puumala hantavirus is one type of hemorrhagic fever with renal syndrome (HFRS). There is considerable variability in the clinical severity of NE. Many infections are subclinical but the disease can even be fatal. We questioned whether the wide spectrum in the outcome of NE is dependent on host-related immunological factors by determining the major histocompatibility complex markers (MHC) in 74 adult patients with NE. Patients with the most severe course of the disease had a very high frequency of HLA B8, C4A*Q0, and DRB1*0301 alleles. HLA B8 was found in all 7 (100%) patients with shock and in 9 of the 13 (69%) patients who required dialysis, versus in only 25 of 74 (34%) in the entire population, and in 14 of 93 (15%) controls. In addition, various other clinical findings pointing to a severe form of NE were found to be associated with these alleles. Interestingly, the same MHC alleles are risk factors for various autoimmune diseases. This is the first study where a certain HLA haplotype is found to be associated with the clinical course of an acute viral disease or acute nephritis.

Nephropathia epidemica (NE), often referred to as a mild form of HFRS, is endemic in Scandinavia, European Russia and in Balkans [1]. About 1000 serological diagnoses of NE are made annually in Finland [2]. As judged from the seroprevalence in Finland (6%), many infections are subclinical or undiagnosed. During recent years hundreds of Puumala hantavirus infections have also been reported in Western Europe [3].

The causative agent, Puumala virus, is a member of the *Hantavirus* genus in the *Bunyaviridae* family. HFRS is caused by several hantavirus serotypes, Hantaan (Korean hemorrhagic fever), Seoul and Dobrava (or Belgrade) virus [1, 4]. The recently described hantavirus, named Sin Nombre virus, mainly causes pulmonary symptoms and is lethal in 55% of patients [5, 6]. The natural hosts of hantaviruses are chronically, but asymptomatically infected rodents and insectivores, which transmit the virus to humans in their excretions. Transmission from human to human has not been reported. Puumala virus is carried by the bank vole, *Clethrionomys glareolus* [7].

NE is clinically characterized by high fever, headache, abdominal pains and hemorrhages [8, 9]. Hypotension is present in 10% of patients [8]. The mortality rate is very low, but a number of lethal cases have recently been reported [10, 11]. The renal involvement results in transient massive proteinuria, hematuria and impairment of renal function [12]. Acute renal failure is present in most hospital-treated patients and transient hemodialysis in needed in a minority [8]. Acute tubulointerstitial nephritis is the typical renal biopsy finding [12–14]. Common laboratory findings are leukocytosis, thrombocytopenia, hypoproteinemia and moderate elevation of liver enzymes, ESR and CRP level. Increased hematocrit (hemoconcentration) is usual on admission and is followed by anemia [8, 9].

The pathogenesis of NE is poorly known. Puumala virus causes no cytopathic effects in cultured cells but has a wide cell susceptibility *in vitro* [15]. The main site of the virus replication in man is not known, but endothelial cells are probable targets. In hantavirus infections, a viral antigen and/or genome has been detected in the endothelium of many tissues. An important feature in hantavirus infections is universally increased capillary permeability [16]. Some NE patients have abnormalities in chest roentgenography due to pulmonary capillary leakage and acute renal failure [8]. This feature bears a similarity to the hantavirus pulmonary syndrome recently described in the United States [6].

It has been suggested that immunological factors are involved in the pathogenesis of hantavirus infections. The T-cell-mediated immune response against acute Hantaan virus infection seems to be important in nude mice [17]. However, increased amounts of CD8-positive T-cells, mainly killer cells, have also been detected during the acute phase of the disease in humans [18]. Little information is available on cell-mediated immune responses in Puumala virus infection.

As persons having certain HLA haplotypes, especially HLA B8 DR3, are known to have an increased or altered immune responsiveness to a variety of antigens [19], we sought to establish whether the tendency to NE, or to some particular symptoms or findings of NE, is determined by these genetic markers.

Methods

The subjects involved were all treated at Tampere University Hospital. The material included 51 males and 23 females, aged from 20 to 75 (mean 43) years. The following previous diseases were noted: essential hypertension in 12, coronary heart disease in 3, type-2 diabetes mellitus and bronchial asthma in 2 each, as well

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Table 1. HLA and complement C4 alleles in seven NE patients suffering from shock

HLA A	HLA B	C4A	C4B	DRB1	DQA1	DQB1
2,25	8,15	3	1	0301,08	0401,0501	0201,04
3,9	7,8	nd[a]	nd[a]	0301,13	0103,0501	0201,0603
1,3	8,15	3	1,1	0301,13	0103,0501	0201,0603
2	5,8	Q0	1,1	0301,15	0101/2,0501	0201,0602
1,28	8	3,Q0	1,2	0301,01	0501,0101/2	0201,0501
1,3	8	Q0	1	0301	0501	0201
1	8,15	3,Q0	1	0301,13	0501,0101/2	0201

[a] not determined since no fresh serum sample was available

Table 2. HLA and complement C4 alleles in 13 NE patients who required dialysis treatment; the first four patients also suffered from shock

HLA A	HLA B	C4A	C4B	DRB1	DQA1	DQB1
2,25	8,15	3	1	0301,08	0401,0501	0201,04
3,9	7,8	nd[a]	nd[a]	0301,13	0103,0501	0201,0603
1,3	8,15	3	1,1	0301,13	0103,0501	0201,0603
2	5,8	Q0	1,1	0301,15	0101/2,0501	0201,0602
1,2	8	Q0	1	0301	0501	0201
1,24	8,40	Q0	1,2	0301,13	0101/2,0501	0201,0604
1,3	7,8	3	1,1	0301,15	0101/2,0501	0602,0201
2,28	8,44	3	1	0301,11	0501	0301,0201
1,3	8,35	nd[a]	nd[a]	01,13	0101/2,0103	0501,0603
2,24	51,44	3,3	1,Q0	04,09	03	0302,0303
3	18,35	3,3	1,Q0	04	03	0302
3	18,35	3	1,Q0	01,l3	0101/2,0103	0501,0603
24,28	35,39	nd[a]	nd[a]	14,08	0101/2,0401	0402,0503

[a] nd, not determined since no fresh serum sample was available

as multiple sclerosis, psychosis, thyroid papillary carcinoma, rheumatoid arthritis and hyperlipidemia in 1 each. Fifty-four (73%) patients had no chronic diseases. Laboratory values were available for all patients except serum complement levels that were measured in 30 patients.

A recent Puumala virus infection was demonstrated in all cases by a fourfold or greater rise in antibody titer against the virus (indirect immunofluorescence antibody test) and/or detection of low avidity of the IgG antibodies to Puumala virus [2].

HLA specimens were gathered in two groups. Samples from randomly selected 49 patients who had been treated for NE in our hospital during years 1985 to 1993 were obtained retrospectively (group A). Another group consisted of 25 consecutive patients with NE during years 1994 to 1995 (group B).

HLA A, B, and Cw alleles were determined by the standard microlymphocytotoxicity test. The DRB1 alleles were determined using group-specific DNA-amplification followed by restriction enzyme digestions [20]. The DQA1 alleles were determined using the PCR-AFLP method of Ota, Seki and Nomura [21]. No distinction could be made between DQA1*0101 and DQA1*0102; thus they were typed as DQA*0101/2. For DQB1 typing, a set of oligonucleotide probes was hybridized to amplified DNA samples as described elsewhere [22]. The validity of the HLA typing results is constantly monitored by participation in the serological and DNA-level reference typings organized by the UCLA tissue typing laboratory. The complement component C4 allotypes were determined essentially as described by Awdeh and Alper [23].

A control group for HLA studies comprised of 93 cadaveric kidney donors. The prevalence of Puumala virus antibodies in this group is not known. The HLA frequencies in our control group (details not shown) did not significantly deviate from those reported earlier in the Finnish population [24].

The results are presented as group means and proportions. In view of the non-normal distribution of variables the differences between group means were tested by Mann-Whitney *U*-test. Fisher's exact test was used for the 2 × 2 tables. The significance of differences in the HLA allele frequencies was also estimated by Fisher's exact test. The *P* values were corrected by multiplying by the number of alleles tested in each locus.

Results

Serological HLA A, B, and Cw, PCR-typed DRB1, DQA1, DQB1 and DPB1 alleles, as well as the HLA-linked complement component C4 allotypes were determined in 74 patients with hospital-treated NE and their frequencies compared with those in the control group. None of the differences in allele frequencies (data not shown) remained statistically significant after correction (that is, multiplying the *P* values by the number of alleles compared in each locus), suggesting that the patient group as a whole showed no strong HLA association. It was, however, of interest that slightly higher frequencies of HLA A1 (28% vs. 16%, $P_{uncorr} = 0.04$), B8 (34% vs. 15%, $P_{uncorr} = 0.01$), and DQB1*0201 (43% vs. 23%, $P_{uncorr} = 0.02$) were found in the patient than in the control group. The frequencies of HLA alleles were similar in patient groups A and B; for example, HLA B8 was found in 16 of 49 (33%) in group A and in 9 of 25 (36%) in group B.

Seven (5 males and 2 females) out of 74 patients were in a clinical shock on admission. The lowest blood pressure in these patients was 80/50, 80/40, 78/58, 70/50, 70/40, 60/40 mm Hg and unmeasurable in one patient. Table 1 shows the HLA alleles in these patients. It is of interest that they all had the HLA B8, DRB1*0301, DQA1*0501, DQB1*0201 alleles. None of the remaining 67 patients had clinical symptoms of hypotension, and the lowest blood pressure measured in them was 90/60 mm Hg.

Table 2 shows HLA alleles in those 13 (9 males and 4 females) who during hospital care required dialysis treatment. Nine (69%) of these patients were HLA B8 positive and 8 (62%) DRB1*0301 positive. Thus all but one of the HLA B8 positive patients in these two subgroups (Table 1 and 2) had the HLA DRB1*0301, DQA1*0501, DQB1*0201 alleles known to be in a strong linkage disequilibrium with HLA B8 [23]. They apparently also carried the C4A'null' allele (C4A*Q0), as can be expected [25] from its strong association with the HLA B8 DRB1*0301 haplotype, although the C4 phenotyping was not informative in all cases. It is thus evident that patients suffering from the most severe outcome of NE frequently carry HLA B8, C4A*Q0, DRB1*0301, DQA1*0501, and DQB1*0201 haplotypes.

There were no differences in the frequency of previous diseases between the shock and dialysis groups and the other patients. Of the patients with clinical shock two had essential hypertension, the other five having previously been in good health. Three of the 13 who were dialyzed had essential hypertension, the other had no previous diseases. The age of the patients in these two subgroups did not differ from that of other patients.

To further study the relevance of this HLA association, a variety of clinical and laboratory findings were evaluated with respect to the HLA B8 and DRB1*0301 positivity (Table 3). A

Table 3. Laboratory findings in HLA B8 and DRB1*0301 positive and negative patients

Finding	HLA B8			HLA DRB1*0301		
	Positive (N = 25)	Negative (N = 49)	P value	Positive (N = 22)	Negative (N = 52)	P value
Creatinine-max *μmol/liter*	639 (409)	319 (278)	<0.001	673 (392)	323 (290)	<0.0001
Urea-max *mmol/liter*	33.0 (17.2)	17.7 (12.7)	<0.001	32.8 (16.8)	18.8 (14.1)	<0.01
Blood leukocyte count-max (× 10^9/*liter*)	18.8 (11.9)	10.3 (3.6)	<0.0001	18.8 (12.3)	10.8 (4.5)	<0.001
Hematocrit-min	0.33 (0.06)	0.37 (0.05)	<0.01	0.33 (0.06)	0.37 (0.05)	<0.01
Hematocrit-max	0.47 (0.11)	0.43 (0.05)	NS	0.47 (0.11)	0.43 (0.05)	NS
Platelet count-min (× 10^9/*liter*)	66 (71)	90 (59)	<0.01	61 (50)	92 (67)	<0.05
Serum protein-min *g/liter*	55 (7)	58 (5)	NS	55 (7)	58 (6)	NS
Serum C3 *g/liter*	0.89 (0.20)	1.05 (0.20)	NS	0.94 (0.16)	1.02 (0.25)	NS
Serum C4 *g/liter*	0.13 (0.05)	0.20 (0.06)	<0.01	0.14 (0.06)	0.19 (0.07)	<0.05

Values are expressed as means (± SD). Abbreviations are: max, maximum; min, minimum. Statistical analysis is by the Mann-Whitney U-test.

Table 4. Clinical and radiologic findings in HLA B8 and DRB1*0301 positive and negative NE patients

Finding	HLA B8			HLA DRB1*0301		
	Positive (N = 25)	Negative (N = 49)	P value	Positive (N = 22)	Negative (N = 52)	P value
Age *years*	45 (10)	42 (12)	NS[b]	44 (8)	43 (13)	NS[b]
Lowest systolic BP[a] *mm Hg*	105 (27)	121 (15)	<0.01[b]	102 (26)	121 (15)	<0.01[b]
Lowest diastolic BP[a] *mm Hg*	66 (15)	75 (11)	<0.05[b]	66 (16)	75 (11)	<0.05[b]
Shock %	28	0	<0.0001[c]	32	0	<0.0001[c]
Abnormal chest roentgenography %	45	15	<0.05[c]	45	16	<0.05[c]
Treatment time at hospital *days*	14 (9)	9 (4)	<0.01[b]	13 (9)	9 (5)	<0.05[b]

Values are expressed as means (± SD) and percentages.
[a] BP, blood pressure
[b] Mann-Whitney U-test
[c] Fisher's exact test

total of 25 of 74 patients were positive for HLA B8 and 22/74 for HLA DRB1*0301. All DRB1*0301 positive patients also had the HLA B8 antigen. The data shown in Table 3 clearly indicate that findings pointing to clinically severe NE were in most cases strongly associated with HLA B8 and DRB1*0301. The result was basically similar whether comparison was made with respect to HLA B8 positivity among the patients, or with respect to DR1*0301. Therefore, our data provide no information as to which part of this haplotype might show the strongest association. No comparisons with other HLA alleles were done. The highest serum creatinine and urea values among HLA B8 positives were 602 (435) μmol/liter and 34.4 (20.6) mmol/liter in group A and 702 (372) μmol/liter and 31.1 (12.3) mmol/liter in group B. The respective values among HLA B8 negatives were 334 (305) μmol/liter and 17.6 (13.3) mmol/liter in group A and 298 (220) μmol/liter and 17.9 (12.1) mmol/liter in group B.

Acute renal failure considered severe (maximal serum creatinine > 500 μmol/liter) was present in 15 (60%) HLA B8 positive and in 7 (14%) HLA B8 negative patients ($P < 0.001$). All HLA B8 positive patients had impairment of renal function (maximal creatinine > 110 μmol/liter), whereas in 8 (16%) of the HLA B8 negatives serum creatinine remained normal throughout hospitalization ($P < 0.0001$).

No differences were observed between HLA B8 and DRB1*0301 positive and negative groups in the highest values of CRP, ESR, alanine aminotransferase, daily urine protein excretion or the amount of hematuria.

The mean values of lowest systolic and diastolic blood pressure observed during hospital care were lower in HLA B8 and DRB1*0301 positive than negative patients (Table 4). About one-third of HLA B8 and DRB1*0301-positive patients suffered from shock whereas and no one without these HLA antigens had this clinical expression (Table 4). Shock was diagnosed in four patients within group A and in three within group B.

Chest roentgenography was taken during hospital care in 63 patients. In 16 (25%) of them abnormalities were observed. These included pleural effusions, parenchymal infiltrations or frank pulmonary edema [8]. Altogether 10 of 22 (45%) HLA B8 and 9 of 20 (45%) DRB1*0301 positive patients had abnormal findings in chest roentenography (Table 4). Pulmonary edema was found in three patients, of whom two were HLA B8 positive.

The time the patients were treated at hospital reflects the clinical severity of the disease. This time was significantly longer in HLA B8 and DRB1*0301 positive than in negative patients (Table 4). The average treatment time in patient group B was 13 days in HLA B8 positives and eight days in HLA B8 negatives. All patients recovered.

The frequency of previous diseases did not differ with respect to the HLA status (HLA B8 or HLA DRB1*0301) of the patients.

Seventy-two percent of the HLA B8 positive and 77% of the HLA DRB1*0301 positive patients had no chronic diseases.

Discussion

The present study reveals that individuals with the HLA B8, C4A*Q0, DRB1*0301, DQA1*0501, DQB1*0201 haplotype have a genetic risk of a severe outcome of Puumala hantavirus-induced nephropathia epidemica (NE). In particular, all patients who suffered from shock had HLA B8 and DRB1*0301. Also, a more severe renal failure was associated with these alleles. Our finding was not explained by a higher frequency of previous diseases in HLA B8 DR*0301 positive patients. We therefore assume that this haplotype is a genuine genetic risk factor. As the HLA B8 DR*0301 haplotype is strongly associated with an abnormal immune response (see below), our results most probably imply that the immune system of the host is involved in the pathogenesis of Puumala virus infection.

The HLA B8 DRB1*0301 haplotype is of special interest in many ways. First, there is a very high linkage disequilibrium between the alleles of this haplotype [25] and the whole HLA region encompassing over 3 Mbp of DNA, inherited usually as a fixed combination. This renders the precise mapping of susceptibility genes within this haplotype difficult, in particular as more than 50 genes have been identified within this segment [26]. Second, the HLA B8 DRB1*0301 haplotype is very strongly associated with many autoimmune diseases, among them systemic lupus, coeliac disease, thyroiditis, Addison's disease, type-1 diabetes mellitus and chronic active hepatitis [27]. Third, carriers of this haplotype are known to evince abnormal *in vitro* responses in various immune assays; for example, they have defects in immune complex clearance and hyperresponsivity in mixed lymphocyte reaction [28]. They also show increased antibody production and decreased suppressor-cell function [29, 30].

The mechanism connecting this HLA haplotype to these findings or to a particular clinical course of NE is unknown. There are no obvious explanations for the wide variation in the severity of renal failure in NE. It does not correlate with the age or the degree of hypotension of the patients [8, 12]. A predominant feature of NE is renal damage, that is, morphologically acute tubulointerstitial nephritis, where tubular epithelial and luminal changes are accompanied by interstitial edema and inflammatory cell infiltrates. The cell infiltrates are predominated by lymphocytes and also include monocytes/macrophages, plasma cells and eosinophilic granulocytes [12–14]. Recently a direct invasion of renal tubules by Hantaan virus was reported [31]. Obviously the virus infection alone cannot explain the tissue injury in NE, since in tissue culture Puumala virus will cause no cytopathic changes [15]. It is therefore conceivable that the immune system of the host may play an important role in the damage. In principle, the host's immune response may be related to a direct killing of infected cells, or there may be an autoimmune-type of response where the originally virus-targeted immunity attacks against cross-reacting self structures [32]. The autoimmunity mechanism is of particular interest since the HLA B8 DRB1*0301 haplotype is strongly associated with autoimmune diseases. Both mechanisms may also function. In the present study HLA B8 and DRB1*0301 positive patients had marked leukocytosis, possibly reflecting an altered immune response.

As the HLA B8, DRB1*0301 haplotype invariably carries a deletion of the C4A gene encoding the C4A component of the complement system [25], one might conceivably attribute significance to the the C4A defect. Defects in the components of the classical pathway have been associated with impaired clearance of immune complexes [33], that certainly may be a crucial factor in certain forms of renal damage. Also, complement activation can be associated with the pathogenesis of vascular dysfunction [16]. Current data on complement activation in NE, however, is limited, but low C4 levels could be observed in HLA B8 and DRB1*0301 positive patients during the acute phase of the disease. Whether these were acquired or genetically determined will require further studies.

Some earlier studies indicate that carriers of HLA B8 DRB1*0301 haplotype would have an altered immunity against viruses. This haplotype, particularly in a homozygous form, causes failure of the response against hepatitis B vaccine [34, 35]. In addition, the same HLA alleles have been found to be associated with a progression to symptomatic AIDS after HIV infection [36]. The present report indicates that individuals with HLA B8 DRB1*0301 haplotype have an increased risk for severe clinical course of Puumala hantavirus infection. It is thus possible that carriers of the HLA B8 DRB1*0301 haplotype are in some respect more prone to defects in the handling of many virus infections.

In conclusion, HLA B8 and DRB1*0301 alleles are associated with a severe clinical course of NE, Puumala hantavirus-induced HFRS. The pathogenetic mechanism underlying this relationship calls for further investigation. This is the first study where a certain HLA haplotype is found to be associated with the clinical picture of an acute viral disease or acute nephritis. Interestingly, the same HLA haplotype is known to be associated with several chronic autoimmune diseases. The authors are of the opinion that HLA studies should also be undertaken of more severe forms of hantavirus diseases such as Korean hemorrhagic fever [1] and hantavirus pulmonary syndrome recently found in the United States [5, 6].

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