Natural clearance of hepatitis C virus in hemophilia patients

Raimonds Simanis, Sandra Lejniece[1], Arturs Sochnevs[2], Jelena Eglite[2], Gunta Chernevska[3], Zhanna Kovalova[4], Dace Gardovska[4], Agita Jeruma, Velga Kuse, Ludmila Viksna

Department of Traditional Infectology, Tuberculosis and AIDS, [1]Department of Internal Diseases, [2]Institute of Immunology, Riga Stradins University, [3]University Children's Hospital, Latvia, [4]Department of Pediatrics, Riga Stradins University, Latvia

Key words: *hepatitis C virus; hemophilia; natural clearance; human leukocyte antigen.*

Summary. *Objective. The objective of this study was to investigate the prevalence of HCV (hepatitis C virus) infection in hemophilia patients in Latvia and to analyze association between natural clearance of HCV and human leukocyte antigen (HLA) class II genes.*

Material and methods. From 61 hemophilic patients participating in this study, 38 were adults and 23 were pediatric patients younger than 18 years. To analyze association between HLA class II alleles and natural clearance of HCV, the gene frequency was compared in hemophilia patients group and the control group of 60 healthy subjects, all men. Serum HCV RNA was qualitatively determined and HLA class II alleles were identified by polymerase chain reaction (PCR) method.

*Results. HCV infection is common among hemophilia patients in Latvia. Antibodies to HCV were found in 45 of 61 (74%) hemophilia patients. In 41% of hemophilia patients (18 of 44), HCV infection resolved spontaneously. Children cleared HCV more frequently than adults (7 of 11 comparing to 11 of 33, respectively; OR=3.50; P<0.05). The frequency difference was found to be statistically significant when comparing HLA alleles distribution in the sample of hemophilia patients who naturally cleared HCV (n=18) and in the control group (n=60) (corresponding frequency of HLA-DRB1*07 allele – 4 (11.11%) and 9 (1.67%); OR=7.38; P<0.05).*

*Conclusions. Natural clearance of HCV infection is frequently found in hemophilia patients in Latvia. Children are more likely to clear virus naturally than adults. There is an association between natural clearance of HCV and HLA allele DRB1*07 in hemophilia patients.*

Introduction

Chronic hepatitis is an important public health problem affecting more than 500 million people worldwide (1) and 80–100 thousand in Latvia (2). According to literature data, hepatitis C virus (HCV) is the major cause of chronic liver disease in adults and children with hemophilia (3). Before the introduction of HCV screening of human blood products, most of children with hemophilia were infected with HCV. In Latvia screening of donated blood for presence of anti-HCV was introduced in November 1993. Cryoprecipitate and fresh-frozen plasma for treatment of hemophilia patients were used until June 2000. Approximately 10% (4) to 50% (5, 6) of patients who were infected with HCV have been shown to clear the infection naturally. Higher rates of spontaneous resolution of HCV infection have been observed in some patient subgroups such as young women after a common-source outbreak of contaminated anti-D immunoglobulin, children infected with HCV while undergoing cardiac surgery or cancer chemotherapy, or in healthcare workers after a needle stick injury (7). The mechanisms underlying the spontaneous viral clearance or development of chronic HCV infection have not been clearly identified yet (8). Apart from viral characteristics (viral genotype, quasispecies distribution, and viral load), it is generally accepted that cellular immune responses play an important role in viral clearance and disease resolution (5).

The outcome of HCV infection is determined by complex host-virus interactions (9) and based on studies involving hemophilia patients; it seems that genetic factors have rather modest influence on the outcome of hepatitis C (7). Nevertheless, immune system, including human leukocyte antigen (HLA) complex, plays an important role in natural clearance of HCV (10) and is worth examining in different populations.

Hemophilia patients who received unsterilized blood products such cryoprecipitate have extremely

Correspondence to R. Simanis, Department of Traditional Infectology, Tuberculosis and AIDS, Riga Stradins University, Linezera iela 3, 1006 Riga, Latvia. E-mail: raimonds.simanis@e-teliamtc.lv

high prevalence of HCV infection reaching 90% in many countries (7, 11). The preliminary analysis showed that Latvia is not an exception in terms of HCV infection prevalence in hemophiliacs. Since modern treatment options are available now, it was important to determine the prevalence of HCV infection among hemophilia patients in Latvia in order to determine those who need treatment and those who cleared virus naturally. By analyzing the group of hemophilia patients who have cleared HCV naturally, possible reasons of this phenomenon were found and described. The study of HCV infection in hemophiliacs has been performed in Latvia for the first time. There are also not so many studies of this kind performed in different countries around the world. The most important study so far, trying to find answers for reasons of natural clearance of HCV and including 257 sibling pairs from 3993 hemophilic patients, has been published recently (7). Our study focused more on one internal factor, namely, some HLA II class alleles, that could contribute to spontaneous resolution of HCV infection in population in Latvia. The results of different studies confirm the negative association between chronic HCV infection and DQB1*0301 allele (12). Association of DQB1*0301 allele with natural clearance of HCV is mentioned by many authors in original studies and reviews (10, 12–23). The other allele described as being important in patients who clear HCV spontaneously is DRB1*1101 (13, 16, 18, 20–24).

The objective of this study was to investigate the prevalence of HCV infection in hemophilia patients in Latvia and to analyze the association between natural clearance of HCV and HLA class II genes.

The another objective was to determine distribution of HCV genotypes in children and adults with hemophilia in Latvia.

Material and methods

Subjects. Serum samples were obtained from 61 patients with hemophilia. Thirty-eight of them were adults and 23 were pediatric patients younger than 18 years. The study was performed in accordance with the principles of Helsinki Declaration and approved by Ethics Committee. All hemophilia patients or their parents (for the group of pediatric patients) signed informed patient consent forms.

To analyze association between HLA class II alleles and natural clearance of HCV, the gene frequency was compared in hemophilia patient group (n=44) and the control group of healthy subjects (n=60, all men). The data on HLA class II alleles of the control group were obtained from the database of the Laboratory of Immunology of the Riga Stradins University.

Laboratory methods. Blood samples were analyzed to detect anti-HCV, HCV RNA, and HCV genotypes. Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to detect antibodies to HCV (AxSYM system HCV version 3.0, Abbott, USA; ORTHO HCV version 3.0, Ortho-Clinical Diagnostics Ltd., USA; INNOTEST HCV Ab IV, Innogenetic, Belgium; MONOLISA anti-HCV PLUS version 2, BIO-RAD, France). Serum HCV RNA was qualitatively determined by a commercially available reverse transcription polymerase chain reaction (PCR) method (AMPLICOR Hepatitis C Virus Test, version 2.0, Roche Molecular Systems, Inc., NJ, USA). HCV genotype was determined using a method of reverse hybridization LiPA (The VERSANT HCV Genotype Amplification Kit (LiPA), Bayer Corporation, Germany). HLA class II alleles were determined by PCR method. DNA isolation was performed as follows: genomic DNA was extracted from proteinase K-treated peripheral blood leukocytes with the routine method of salt off. The DNA was stored in TE buffer (10 mL Tris-HCl, pH 7.5, and 2 mL 0.5 M Na_2 EDTA/L d-H_2O). We used DNA obtained stamps for genotyping or storing in –20°C. DNA concentration was determined by fluorescent method with DNA fluorimeter and was 100–200 μg/mL in the middle. Dry DNA and resuspend in TE buffer (25). HLA-DR and -DQ Genotyping by PCR: low-resolution HLA-DR typing for DRB1*01 through 18 as well as for DQB1*0201-202, *0301-305, *0401-402, *0501-504, *0601-608 was performed by PCR method with amplification with sequence-specific primers (PCR-SSP). The reaction mixture (15 μL) contained: 1 μL DNA, 1.5 μL PCR buffer [50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-Cl, (pH 8.3)], 0.6 μL dNTPs (25 mmol/L), 1.0 μL specific primers (0.2 mol/L), and 0.5 U of the Taq DNA polymerase (Promega). In addition, the internal positive control primer pair, C3 and C5, was included in all reaction mixtures at a concentration of 5-fold lower than the allele- and group-specific primers. The reaction mixture was subjected to 35 amplification cycles (Program the Thermo-Cycler DNA-technology, Russia), each consisting of denaturation at 94°C (60 s), followed by one cycle, annealing at 94°C (20 s), 67°C (2 s) followed by seven cycles and extension at 93°C (5s), 65°C (4s) with a final extension step 28 cycles. PCR products were visualized by agarose gel electrophoresis. After addition of 2 M loading buffer, the PCR reaction mixtures were loaded in agarose gels pre-stained with ethidium bromide (0.5 μk/mL gel). Gels were run for

15 min at 10 V/cm gel in 0.5 mM TBE buffer, then examined under UV illumination, and documented (25, 26).

Statistical analysis. The distribution of HLA-DRB1*, HLA-DQA1*, and HLA-DQB1* alleles in hemophilia patients and control group subjects was compared using Pearson's chi-square (χ^2) test with Mantel-Haenszel correction or Fisher's exact probability test when expected value was less than 5. P values less than or equal to 0.05 were regarded as statistically significant. Odds ratios (ORs) were used in place of relative risk. SPSS statistical software version 14 and Microsoft Excel 2003 were used.

Results

HCV infection in hemophilia patients. HCV infection is common among hemophilia patients in Latvia. Antibodies to HCV were found in majority of hemophilia patients (Table 1). HCV infection is more frequently found in adults (89% or 34 of 38) than in pediatric patients (48% or 11 of 23). Twenty-three children, born between 1987 and 2004, were included in the study. All children who cleared virus spontaneously were born during the period from 1990 to 1994. From all hemophilia patients with positive antibodies to HCV, persistent hepatitis C was determined in 26 (58%) patients. In one patient, sustained viral response was achieved by monotherapy with interferon-alpha before this study. This patient was excluded from calculations when assessing the frequency of natural clearance of HCV.

HCV genotype distribution. Hepatitis C virus genotypes 1b, 3a, and 2 were found in 16 (61%), 8 (31%), and 2 (8%) patients, respectively. Since there are no precise data on distribution of HCV genotypes in Latvian population, comparison with different patient groups (27, 28) was made (Table 2).

Natural clearance of HCV. In 41% (18 of 44) of hemophilia patients, HCV infection resolved spontaneously. Children cleared HCV more frequently than adults (7 of 11 (63.63%) comparing to 11 of 33 (33.33%), respectively; OR=3.50; P<0.05). Adults older than 40 years cleared HCV more often as compared to those younger than 40 years (5 of 10 (50%) comparing to 6 of 24 (25%), respectively; OR=3.00; P<0.23). Differences between frequencies of natural clearance did not reach statistical significance due to a small number of hemophilia patients.

HLA class II alleles in hemophilia patients in Latvia. The frequency difference was found to be statistically significant when comparing the distribution of HLA class II alleles in hemophilia patient group (n=60) and control group (n=60): HLA-DRB1*05 (corresponding number of alleles – 32 (26.67%) and 17 (14.17%)), HLA-DRB1*07 (corresponding number of alleles – 16 (13.33%) and 2 (1.67%)) and HLA-DQB1*0502-4 (corresponding number of alleles – 14 (11.67%) and 4 (3.33%)), Table 3.

Table 1. **Prevalence of HCV infection in patients with hemophilia in Latvia**

Category	Adults n (%)	Children n (%)	All patients n (%)
Positive anti-HCV	34 (89)	11 (48)	45 (74)
Negative anti-HCV	4 (11)	12 (52)	16 (26)
Total	38	23	61

Anti-HCV – antibodies to hepatitis C virus.

Table 2. **Distribution of HCV genotype in different patient groups**

HCV genotype	Hemophilia patients in USA n (%) (n=31)	Hemophilia patients in Latvia n (%) (n=26)	Chronic hepatitis C patients in Lithuania n (%) (n=1097)
1a	10 (32)	0	0
1b	4 (13)	16 (61.5)	678 (61.8)
2	2 (6)	2 (7.7)	100 (9.1)
3	13 (43)	8 (30.8)	319 (29.1)
4a	1 (3)	0	0
Mixed	1 (3)	0	0

There are reports about association of HLA class II alleles with inhibitor formation to coagulation factor VIII (29). In our study, there was no statistically significant difference between groups of alleles related to inhibiting antibody formation to coagulation factor VIII: HLA-DQA1*0102, HLA-DQB1*0602-8, and HLA-DRB1*01.

HLA class II alleles in hemophilia patients with natural clearance of HCV. The frequency difference was found to be statistically significant when comparing the distribution of HLA alleles in the sample of hemophilia patients who naturally cleared HCV (n=18) and in the control group (n=60): HLA-DRB1*07 (corresponding frequency of allele – 4 (11.11%) and 9 (1.67%)), Table 4. There was no statistically significant difference in frequency distribution of allele DQB1*0301 between hemophilia patients and control group.

HLA class II alleles in pediatric hemophilia patients with natural clearance of HCV. Because of the very high number of children, who have cleared HCV naturally, we undertook comparison of HLA II class genes of these children (n=7) with the control group of 60 healthy subjects. The frequency difference was found to be statistically significant when comparing HLA allele distribution in the sample of hemophilia patients – children who naturally cleared HCV (n=7) and in the control group (n=60): HLA-DRB1*03 (frequency of the allele correspondingly 21.43% and 5%) and DQB1*0401-2 (21.43% and 3.3%), Table 5.

In hemophiliacs – children with persistent hepatitis C – HLA alleles DRB1*07 (25% and 1.67%; OR= 19.67; P<0.0003) and DQA1*0401 (12.50% and 1.67%; OR=8.43; NS) were found more frequently when comparing with the control group.

Discussion

In comparison to the literature data, where approximately 20% of patients have been shown to clear HCV infection naturally, in our report it reaches 64% in pediatric group. In adult hemophilia patients in Latvia, natural clearance of HCV virus shown in our study is 33%.

It is possible and has been also confirmed by other

Table 3. **Frequencies of HLA alleles in hemophilia patients and controls**

HLA allele	Number of alleles in hemophilia patients n (%) (n=120)	Number of alleles in control group n (%) (n=120)	P value	OR
DRB1*05	32 (26.67)	17 (14.17)	0.02	2.20
DRB1*07	16 (13.33)	2 (1.67)	0.0006	9.08
DQB1*0502-4	14 (11.67)	4 (3.33)	0.0145	3.83

Only statistically significant results are displayed.

Table 4. **Frequencies of HLA allele in hemophilia patients with natural clearance of HCV and controls**

HLA allele	Number of alleles in hemophilia patients n (%) (n=36)	Number of alleles in control group n (%) (n=120)	P value	OR
DRB1*07	4 (11.11)	9 (1.67)	0.026*	7.38

*Fisher exact two-sided test. Only statistically significant results are displayed.

Table 5. **Frequencies of HLA alleles in pediatric hemophilia patients with natural clearance of HCV and controls**

HLA allele	Number of alleles in hemophilia patients n (%) (n=14)	Number of alleles in control group n (%) (n=120)	P value	OR
DRB1*03	3 (21.43%)	6 (5.00%)	0.02	5.18
DQB1*0401-2	3 (21.43%)	4 (3.33%)	0.025*	7.91

*Fisher exact two-sided test. Only statistically significant results are displayed.

studies that patient age at the time of infection is a positive factor that influences clearance from HCV infection. It seems that immune response of young patients is more effective.

The findings in this small study are consistent with HCV genotype 1b prevalence in general population in Latvia although there are no large studies available. It is impossible to determine which HCV genotypes were involved in those cases where natural clearance had occurred. Possibly those were genotypes 2 and 3a. Although genotype 3a is more frequently found in hemophilia patients in the USA, comparing to general population, it seems to be the same (approximately 30%) in both groups in the Baltic States.

There are many factors – external (HCV genotype, route of infection) and internal (genetic) – that contribute to the outcome of HCV infection.

Multiple transfusions of infected blood product also could boost host immune responses against HCV. Hemophilia patients are likely to have been re-infected several times with different HCV genotypes and HCV quasispecies. The mechanism of strong antiviral response in those hemophilic patients, who, despite multiple exposures to virus, cleared HCV successfully, is not clearly investigated and understood.

The fact that among adult hemophilia patients older than 40 years, HCV cleared more often comparing to those younger than 40 years also leads to thoughts that long-term multiple exposure to the virus could contribute to more successful results in the fight between host and pathogen. Since differences between the groups did not reach statistical significance, it would be important to conduct studies in bigger countries, involving a greater number of hemophilia patients.

There were no statistically significant differences observed between frequency distribution in hemophilia patients who cleared HCV naturally and control groups of alleles, more frequently described in the literature in association with HCV clearance – DQB1*0301 and DRB1*1101. The allele DRB1*07 is described in literature in association with persistent HCV infection (21). Indeed, in our study, DRB1*07 was also quite often found in the hemophilia patients with chronic hepatitis C. At the same time, there is an association observed between allele DRB1*07 and successful response to interferon-alpha treatment (30). Much more complex immune response mechanisms are involved in clearance of HCV and, even if the association is found between a single allele and different outcomes of HCV infection, this fact should be addressed with care.

It is unlikely that predicting the outcome of HCV infection based on HLA class II gene frequency will be clinically useful in the closest future. Extremely high polymorphism of HLA class II genes is observed in different populations and patient groups.

Incidence of HLA class II alleles, known to be related to formation of inhibitors to coagulation factor VIII, was even lower in hemophilia patients group comparing to healthy subjects. There is possibly less risk of developing inhibitor antibodies to factor VIII used for treatment in hemophilia patients in Latvia.

Studies of the association between HLA complex and different outcomes of HCV infection should be performed in larger groups in the future, taking into account the results from the smaller studies in different patient groups and populations. Planning of these studies has to be performed with caution, since it is already known based on studies in hemophilia patients with HCV infection that genetic factors are complex and play only a modest role in spontaneous clearance of HCV and other outcomes of HCV infection.

Conclusions

Natural clearance of HCV infection is frequently found in hemophilia patients in Latvia. Persons more likely to clear virus naturally are children and those adult hemophilia patients with a long history of treatment with blood products. There is an association between natural clearance of HCV infection and HLA allele DRB1*07 in hemophilia patients in Latvia. In children, there is an association between natural clearance of HCV infection and HLA alleles DRB1*03 and DQB1*0401-2. These alleles could contribute to successful clearance of HCV infection.

Natūralus hepatito C viruso išnykimas organizme žmonių, sergančių hemofilija

Raimonds Simanis, Sandra Lejniece[1], Arturs Sochnevs[2], Jelena Eglite[2], Gunta Chernevska[3], Zhanna Kovalova[4], Dace Gardovska[4], Agita Jeruma, Velga Kuse, Ludmila Viksna

Rygos Stradins universiteto Tradicinės infektologijos, tuberkuliozės ir AIDS katedra, [1]Vidaus ligų klinika, [2]Imunologijos institutas, [3]Latvijos universiteto vaikų ligoninė, [4]Rygos Stradins universiteto Pediatrijos skyrius, Latvija

Raktažodžiai: hepatito C virusas, hemofilija, natūralus išnykimas, žmogaus leukocitų antigenai.

Santrauka. *Tyrimo tikslas*. Ištirti hepatito C viruso (HCV) infekcijos paplitimą tarp Latvijos ligonių, sergančiųjų hemofilija, ir išanalizuoti natūralaus HVC infekcijos išnykimo ir žmogaus leukocitų II klasės antigenų genų sąsajas.

Medžiaga ir metodai. Tyrime dalyvavo 61 ligonis, sergantis hemofilija, 38 suaugusieji ir 23 vaikai iki 18 metų. Buvo lyginamas genų dažnis tarp ligonių, sergančių hemofilija (n=18), ir sveikų asmenų kontrolinės grupės (n=60, visi vyrai) sąsajoms tarp žmogaus leukocitų II klasės antigenų alelių ir natūralaus HCV išnykimo nustatyti. HCV RNR kiekis serume ir žmogaus leukocitų II klasės antigenų alelės nustatyti polimerazės grandininės reakcijos metodu.

Rezultatai. HCV infekcija yra paplitusi tarp Latvijos ligonių, sergančių hemofilija. Antikūnų prieš HCV rasta 45 (74 proc.) iš 61 sergančio hemofilija ligonio. Savaime HVC infekcija išnyko 41 proc. hemofilija sergančiųjų (18 iš 44). HCV dažniau išnyko vaikų organizme nei suaugusiųjų (7 iš 11 palyginus su 11 iš 33; ŠS=3,50; p<0,05). Lyginant žmogaus leukocitų antigenų alelių pasiskirstymą tarp hemofilija sergančių ligonių, kurių organizme HCV infekcija natūraliai išnyko (n=18), ir kontrolinės grupės tiriamųjų (n=60), nustatytas statistiškai reikšmingas dažnio skirtumas (DRB1*07 alelės dažnis buvo 4 (11,11 proc.) ir 9 (1,67 proc.), atitinkamai; ŠS=7,38; p<0,05).

Išvados. Natūralus HCV infekcijos išnykimas dažnai pastebimas Latvijos ligonių, sergančių hemofilija, organizme. Natūralus viruso išnykimas dažniau pastebimas vaikų organizme nei suaugusiųjų. Nustatyta sąsaja tarp natūralaus HCV išnykimo ir žmogaus leukocitų antigenų DRB1*07 alelės ligonių, sergančių hemofilija, organizme.

Adresas susirašinėti: R. Simanis, Department of Traditional Infectology, Tuberculosis and AIDS, Riga Stradins University, Linezera iela 3, 1006 Riga, Latvia. El. paštas: raimonds.simanis@e-teliamtc.lv

References

1. Foster GR, Goldin RD. Management of chronic viral hepatitis. 2nd ed. Taylor & Francis Group; 2005.
2. Vīksna L. Vīrushepatīts C – norise, ārstēšana, prognoze, profilakse. (Viral hepatitis C – course, treatment, prognosis, prophylaxis.) Nacionālais apgāds; 2003.
3. Blanchette VS, Vorstman E, Shore A, Wang E, Petric M, Jett BW, et al. Hepatitis C infection in children with hemophilia A and B. Blood 1991;78(2):285-9.
4. Yee TT, Griffioen A, Sabin CA, Dusheiko G, Lee CA. The natural history of HCV in a cohort of haemophilic patients infected between 1961 and 1985. Gut 2000;47:845-51.
5. Neumann-Haefelin C, Blum HE, Chisari FV, Thimme R. T cell response in hepatitis C virus infection. J Clin Virol 2005;32:75-85.
6. Gerlach JT, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, et al. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology 2003;125:80-8.
7. Fried MW, Kroner BL, Preiss LR, Wilhelmsen K, Goedert JJ. Hemophilic siblings with chronic hepatitis C: familial aggregation of spontaneous and treatment-related viral clearance. Gastroenterology 2006;131:757-65.
8. Sun J, Li K, Shata MT, Chan TS. The immunologic basis for hepatitis C infection. Curr Opin Gastroenterol 2004;20:598-602.
9. Aberle J, Formann E, Steindl-Munda P, Weseslindtner L, Gurguta C, Perstinger G, et al. Prospective study of viral clearance and CD4+ T cell response in acute hepatitis C primary infection and reinfection. J Clin Virol 2006;36:24-31.
10. Yee LJ. Host genetic determinants in hepatitis C virus infection. Genes Immun 2004;5:237-45.
11. Silonova G, Graudiņa Ž. Vīrushepatīts C. (Viral hepatitis C.) Latvijas Ārsts 1994;1:42-3.
12. Wawrzynowicz-Syczewska M, Underhill JA, Clare MA, Boron-Kaczmarska A, McFarlane IG, Donaldson PT. HLA class II genotypes associated with chronic hepatitis C virus infection and response to alpha-interferon treatment in Poland. Liver 2000;20(3):234-9.
13. Thio CL, Carrington M, Marti D, O'Brien SJ, Vlahov D, Nelson KE, et al. Class II HLA alleles and hepatitis B virus persistence in African Americans. J Infect Dis 1999;179:1004-6.
14. Thio CL, Thomas DL, Goedert JJ, Vlahov D, Nelson KE, Hilgartner MW, et al. Racial differences in HLA class II associations with hepatitis C virus outcomes. J Infect Dis 2001; 184:16-21.
15. Mangia A, Gentile R, Cascavilla I, Margaglione M, Villani MR, Stella F, et al. HLA class II favors clearance of HCV infection and progression of the chronic liver damage. J Hepatol 1999;30:984-9.
16. Thursz M, Yallop R, Goldin R, Trepo C, Thomas HC. Influence of the MHC Class II genotype on outcome of infection with hepatitis C. The Lancet 1999;354:2119-24.
17. Zavaglia C, Martinetti M, Silini E, Bottelli R, Daielli C, Asti M, et al. Association between HLA class II alleles and protection from or susceptibility to chronic hepatitis C. J Hepatol 1998;28:1-7.
18. Nowakowska B, Kacprzak-Bergman I. Zwiazek antygenów zgodności tkankowej HLA z zakazeniem wirusem zapalenia watroby typu C (HCV). (Association between HLA antigens and hepatitis C virus (HCV) infection.) Postepy Hig Med Dosw (Online) 2004;(58):458-62.
19. Donaldson PT. Genetics of liver disease: immunogenetics and disease pathogenesis. Gut 2004;53:599-608.
20. Alric L, Fort M, Izopet J, Vinel JP, Charlet JP, Selves J, et al. Genes of the major histocompatibility complex class II influence the outcome of hepatitis C virus infection. Gastroenterology 1997;113:1675-81.
21. Alric L. Study of host- and virus-related factors associated with spontaneous hepatitis C virus clearance. Tissue Antigens

2000;56:154-8.
22. Cramp ME, Carucci P, Underhill J, Naoumov NV, Williams R, Donaldson PT. Association between HLA class II genotype and spontaneous clearance of hepatitis C viraemia. J Hepatol 1998;29:207-13.
23. Minton EJ, Smillie D, Neal KR, Irving WL, Underwood JC, James V. Association between MHC class II alleles and clearance of circulating hepatitis C virus. Members of the Trent Hepatitis C Virus Study Group. J Infect Dis 1998;178:39-44.
24. Fanning LJ, Levis J, Kenny-Walsh E, Wynne F, Whelton M, Shanahan F. Viral clearance in hepatitis C (1b) infection: relationship with human leukocyte antigen class II in a homogeneous population. Hepatology 2000;31:1334-7.
25. Erlich H, Bugawan T, Begovich A, Scharf S, Griffith R, Saiki R, et al. HLA-DR, DQ and DT typing using PCR amplification and immobilized probes. Eu J Immunogen 1991;18:33-5.
26. Walsh PS, Erlich H, Higuchi R. Preferential PCR amplification of alleles: mechanisms and solutions. PCR Methods and Applications 1992;1:241-50.
27. Eyster ME, Hatzakis A. HCV genotypes in multitransfused hemophiliacs. Gastroenterology 1996;110:A1187.
28. Stikleryte A, Norder H, Abrutiene J, Magnius LO, Ambrozaitis A. Prevalence of hepatitis C virus (HCV) genotypes in Lithuania. 7th Nordic-Baltic Congress of Infectious Diseases Abstracts. 2006. p. 12.
29. Oldenburg J, El-Maarri O, Schwaab R. Inhibitor development in correlation to factor VIII genotypes. Hemophilia 2002; 8(Suppl 2):23-9.
30. Jiao J, Wang JB. Hepatitis C virus genotypes, HLA-DRB alleles and their response to interferon-alpha and ribavirin in patients with chronic hepatitis C. Hepatobiliary Pancreat Dis Int 2005;4:80-3.

Received 18 April 2007, accepted 21 June 2007
Straipsnis gautas 2007 04 18, priimtas 2007 06 21