KIR/HLA ligands immunogenetics markers associated with outcome of hepatitis B virus infection in the Bulgarian population

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Background. Hepatitis B virus (HBV) infection is one of the most common infections worldwide, having negative impact on world health due to the tendency for chronification with late complications such as liver cirrhosis and hepatocellular carcinoma. Natural killer (NK) cells as part of innate antiviral defense influence the clinical course of HBV infection: elimination of the virus or chronic disease.

Aim. Therefore, we investigated the polymorphisms of the main gene systems, regulating NK-cell function: killer cell immunoglobulin-like receptors (KIRs) and their appropriate HLA class I ligands in 144 HBV infected patients (124 chronic carriers and 20 spontaneously recoved) and 126 ethnically matched healthy controls from the Bulgarian population in a case-control study.

Methods. KIRs and HLA ligands were determined by PCR-SSP or PCR high-resolution typing methods.

Results. KIR2DL5B allele variant was significantly less frequent in spontaneously recovered (SR) patients compared to healthy controls (10.0% vs. 45.5%, P^{corr}=0.006). The presence of KIR3DL1*004 allele was higher in chronic HBV carriers (CH) than in controls (33.1% vs. 17.6%, P^{corr}=0.036). Additionally, SR patients differed from healthy individuals by the lower frequency of HLA-Bw4^{Ile80} group ligands (30.0% vs 63.7%, *P*=0.015). Three KIR genotypes were found more frequent in healthy in comparison with HBV infected individuals: ID2 (13.5% vs 5.6%, *P*=0.025), KIR genotype containing 6 activating KIRs (18.0% vs 7.6%, *P*=0.017), and KIR genotype composed of 4 activating and 5 inhibitory KIRs (23.8% vs 5.6%, *P*=0.001).

Conclusion. These data suggest that inherited KIR and HLA class I ligand polymorphisms may influence the clinical course of HBV infection.

Key words: killer immunoglobuline-like receptors, KIR HLA ligands, hepatitis B infection

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INTRODUCTION

Viral hepatitis is one of the most common diseases worldwide. According to the World Health Organization approximately 2.5 billion people have been infected with Hepatitis B virus (HBV), and approximately 3.5% of the global population, or 257 million people are chronic carriers of the virus[1,2]. In Bulgaria, the frequency of the HBV carriers is 3.8%, ranging dependent on the district from 1.9% to 5.3% (ref.[3]), which places the country in the moderate endemicity regions.

The high distribution of the HBV infection, the severity of the disease and the associated late complications and mortality, define HBV as one of the major health and social problems in the modern society. Although the etiologic agent of HBV infection has been known for more than 50 years, the pathogenesis of chronic HBV infection remains unknown. It is considered that HBV is not directly cytopathogenic. The host antiviral immune response may lead both to the removal of the virus, as well as the destruction of the infected hepatocytes and a liver failure. In a chronic infection the effect on the liver is mainly regulated by the host immune system and related cellular immune responses.

Natural killer (NK) cells are the main population of the non-specific cellular immune defense and their function is to destroy virus-infected and transformed cells, and thus to prevent the advance of the liver damage and the occurrence of hepatocellular carcinoma[4-6]. NK cell function is controlled by a complex system of interactions between diverse inhibitory and activating receptors and ligands whose expression is genetically predetermined. In this sense the most well-known and relevant NK cell receptors are those of the immunoglobu-

lin superfamily (Killer immunoglobulin-like receptors – KIRs) and their ligands[7-11]. Currently 15 KIR genes are decribed (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1, KIR3DS1, KIR3DL2, KIR3DL3) and two pseudogenes (*KIR2DP1* and *KIR3DP1*), which encode the inhibitory – 2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B, 3DL1, 3DL2, 3DL3 and the activating – 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DS1 KIR receptors[12]. KIR genes have been divided into two haplotypes A and B depending on the presence of specific genes[12].

Ligands for most KIRs are specific motifs of the human leukocyte antigen (HLA) class I molecules. Dimorphism in the HLA-C α1 domain, defines two HLA-C groups – C1 (Ser77/Asn80) and C2 (Asn77/Lys80), which are ligands for two different receptors of the KIR2DL and KIR2DS receptor groups[8]. HLA-B allotypes with Bw4 motif in position 77-83 are ligands for the inhibitory KIR3DL1 (ref.[9]). The binding affinity between receptor and ligand depends on the amino acid residue at 80th position in the HLA molecule and is stronger when HLA-Bw4 alleles have isoleucine (Ile80) compared to those with threonine (Thr80) at this position[10]. KIR3DL1 recognizes certain HLA- A alleles with Bw4 motifs as well[11]. Consequently, the functional activity of KIRs and the activating or inhibiting signals which they transmit would be dependent on the KIRs/KIR HLA class I ligands genotype of the individual. Recently, considerable data from case-control studies demonstrate the hypothetical role of KIRs and their HLA ligands as genetic factors involved in viral clearance[4,5,13-21].

Therefore, we studied a complex of immunogenetic factors related to KIRs and their HLA class I ligands in infected and non-infected with HBV individuals from the Bulgarian population with the aim to reveal a potential effect of immunogenetic components on the outcome of HBV infection. Better understanding on the mechanisms that govern antiviral immune responses of the host in relation to the genetic diversity would provide opportunities to improve and personalize therapeutic interventions in HBV infected patients.

MATERIAL AND METHODS

Study population

One hundred twenty-four patients with chronic hepatitis B (86 males and 38 females; 44±27 years old, ranged from 18 to 73 years) and twenty individuals spontaneously recovered from acute HBV infection (11 males and 9 females; 44±13.5 years; ranged from 31 to 58 years old) were included in the study. The patients were diagnosed and treated at the Department of Gastroenterology, Clinic of Propaedeutics in Internal Diseases, University Hospital "Alexandrovska" and at the Clinic of Infectious Diseases, Military Medical Academy, Sofia, Bulgaria. Patients were classified in two groups – chronic HBV carriers (HBsAg positive, anti-HBs negative, anti-HBc positive) and spontaneously resolved HBV infection (HBsAg negative, anti-HBs positive, anti-HBc positive) after clinical and laboratory evaluation according to predefined inclusion and exclusion criteria previously described in detail[22]. The healthy controls consisted of 126 randomly selected unrelated individuals (67 females and 59 males, aged 46.6±11.9 years) from the Bulgarian population, without any family history of inherited diseases or any medical history of viral hepatitis, HIV infection, and other diseases such as diabetes, malignancy or autoimmune diseases. These subjects were with normal liver function and negative serological markers for viral hepatitis.

Written and informed consent was obtained from patients and healthy controls. The study was approved by the local ethics committee and National Scientific Fund (DTK02/12-2009).

Genotyping

DNA was extracted from peripheral blood by iPrep PureLink® gDNA™ Blood kit and iPrep™ Purification instrument (Invitrogen, USA).

KIR genotyping was performed by PCR-SSP method with 24 locus specific primers using commercially available Olerup SSP® KIR typing kit (Olerup SSP AB, Sweden). The results obtained were interpreted using the worksheet provided by the manufacturer which allows detection of 16 KIR genes and pseudogenes, differentiation of KIR2DL5A, KIR2DL5B, KIR3DL1*004 alleles and two groups KIR2DS4 alleles (group 1 – KIR2DS4*001 from group 2 – KIR2DS4*003/004/006/007). Two approaches were used for KIR HLA ligands determination – direct typing and/or indirectly. The direct genotyping was performed with commercially available Olerup SSP® KIR HLA ligand typing kit (Olerup SSP AB, Sweden) which distinguishes HLA-C1, -C2, -Bw4^{Ile80}, -Bw4^{Thr80} and -A^{Bw4+} ligand groups. The indirect determination used KIR ligand calculator[23] based on high resolution typing of HLA-A, -B, -C alleles (AlleleSEQR HLA-A, -B and -C PCR/Sequencer Kit, Atria Genetics, USA) as previously described[24].

KIR haplotypes and genotypes were defined in accordance with the allele frequency net database (AFND) (ref.[25]).

Statistical analysis

The frequencies of KIRs, KIR haplotypes, genotypes, KIR HLA class I ligands, and KIR/HLA ligand combinations were determined by direct counting. The comparative analysis between the different groups was performed by Pearson's chi-square test and Fisher exact test. Statistical analysis was done using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL). All *P*-values less than 0.05 were considered to be statistically significant. Bonferroni-adjusted significance test was applied for pairwise comparisons in case of multiple number of comparisons. The corrected p-value (P^{corr}) was obtained multiplying the uncorrected p-value by the number of comparisons made. Odds ratio (OR) and 95% Confidence Intervals (CI) were calculated to variables with significant

Table 1. KIR genes distribution in HBV infected patients and healthy controls.

KIR gene	HC (n=126) n (%)	CHB (n=124) n (%)	SRI (n=20) n (%)
3DL2; 3DL3; 3DP1	126 (100)	124 (100)	20 (100)
2DL4	125 (99.2)	124 (100)	20 (100)
2DP1	122 (96.8)	123 (99.2)	20 (100)
2DL1	118 (93.7)	119 (96.0)	19 (95.0)
2DL2	76 (60.3)	78 (62.9)	14 (70.0)
2DL3	108 (85.7)	111 (89.5)	19 (95.0)
2DL5	82 (65.1)	67 (54.0)	10 (50.0)
2DL5A	42 (34.7) [#]	45 (36.3)	7 (35.0)
2DL5B	40 (45.5)	42 (33.9)	2 (10.0) *P*=0.003; P^{corr}=0.006 OR 0.13[0.02-0.63]
2DS1	57 (45.2)	53 (42.7)	6 (30)
2DS2	82 (65.1)	77 (62.1)	14 (70.0)
2DS3	48 (38.1)	42 (33.9)	4 (20.0)
2DS4	116 (92.1)	116 (93.5)	20 (100)
2DS4norm	18 (20.2) [##]	35 (28.5)	8 (40.0)
2DS4del	70 (78.7) [##]	107 (86.3)	18 (90.0)
2DS5	48 (38.1)	40 (32.3)	6 (30.0)
3DS1	60 (47.6)	47 (37.9)	6 (30.0)
3DL1	114 (90.5)	114 (91.9)	20 (100)
3DL1*004	13 (17.6) [§]	41 (33.1) *P*=0.018; P^{corr}=0.036 OR 2.3[1.1-4.7]	5 (26.3)

HC – healthy controls, CHB – chronic hepatitis B, SRI – subjects with spontaneously resolved HBV infection, KIR – Killer immunoglobulin-like receptor, OR – odds ratio,
[ ] 95% Confidence Interval, [#] n=85, [##] n=89, [§] n=74, P^{corr} – Bonferroni-corrected *P*-values.

differences in order to estimate the associations between genetic factors and HBV infection.

RESULTS

KIR genes/pseudogenes

The distribution of KIR genes in patients and healthy controls is presented in Table 1.

Our data demonstrated that the frequencies of KIR genes were not significantly different between patients' groups and controls, except of KIR2DL5B and KIR3DL1*004 alleles. KIR2DL5B was found in lower frequency in both subgroups of patients compared to unrelated healthy individuals but the difference was statistically significant only for the patients with resolved HBV infection. A higher frequency of the functionally inert variant of the KIR3DL1 gene (KIR3DL1*004) was found among all HBV infected compared to controls, the difference being significant for the chronic hepatitis B subgroup.

To determine whether there were associations with the risk of chronicity of HBV infection, a comparison of KIR frequencies between the two subgroups of patients – chronic carriers and spontaneously resolved infection was made, assuming that both cohorts were exposed to a common environmental factor – hepatitis B virus. The analysis applied showed no significant differences.

KIR genotypes

Forty-one different genotypes were determined according to the presence/absence of individual KIRs among the patients and healthy controls, whose characteristics and frequencies are presented in Table 2. The comparison between the two groups showed lower frequency of KIR genotype ID2 [*P*=0.025; OR 0.38 (95% CI 0.14-0.85)] in the patients' group.

No statistically significant differences were found between HBV infected patients and healthy controls according to the distribution of KIRA and KIRB haplotypes and their combinations. KIR profiles determined by the number of activating and inhibitory KIR genes were also analyzed (data not shown). KIR profile containing six activating genes was more frequent in controls compared to patients [18.0% vs. 7.6%, *P*=0.017; OR 0.4 (95% CI 0.2-0.9)].

Additionally, the ratio between the number of activating and inhibitory KIR genes was calculated for all individuals according to the model of Karabon et al.[26]. Ratios ranged from 0.17 (prevalence of inhibitory KIR genes) to 1.20 (overrepresentation of activating KIR genes, data not shown). KIR genotype with ratio 0.2 (1 activating KIR/5 inhibitory KIRs) was the most frequent both in HBV infected and healthy individuals with a slightly higher incidence in the patients. KIR genotype corresponding to ratio 0.67 (4 activating/6 inhibitory genes) was significantly less frequent in patients compared to controls [5.6% vs. 23.8%; *P*=0.001; OR 2.5 (95% CI 0.98-6.5)].

Table 2. KIR genotypes distribution in HBV infected individuals and healthy controls.

Patients n (%)	HC n (%)	ID*	KIR haplotype combination	*3DL1*	*2DL1*	*2DL3*	*2DS4*	*2DL2*	*2DL5*	*3DS1*	*2DS1*	*2DS2*	*2DS3*	*2DS5*	*2DL4*	*2DP1*	*3DP1*	*3DL2*	*3DL3*
35 (24.3)	22 (17.5)	1	AA	■	■	■	■	□	□	□	□	□	□	□	■	■	■	■	■
27 (18.6)	14 (11.1)	4	Bx	■	■	■	■	■	□	□	□	■	□	□	■	■	■	■	■
13 (9.0)	17 (13.5)	5	Bx	■	■	■	■	■	■	□	□	■	■	□	■	■	■	■	■
11 (7.6)	6 (4.76)	3	Bx	■	■	■	■	■	■	■	■	■	□	■	■	■	■	■	■
8 (5.6)	6 (4.76)	6	Bx	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
8 (5.6)	17 (13.5)	2#	Bx	■	■	■	■	□	■	■	■	□	□	■	■	■	■	■	■
6 (4.2)	4 (3.2)	7	Bx	■	■	■	■	■	■	■	■	■	■	□	■	■	■	■	■
4 (2.8)	1 (0.79)	9	Bx	■	■	■	■	■	■	□	■	■	□	■	■	■	■	■	■
3 (2.1)	5 (3.97)	71	Bx	■	■	□	■	■	■	□	□	■	■	□	■	■	■	■	■
3 (2.1)	3 (2.38)	69	Bx	□	■	■	□	□	■	■	■	□	□	■	■	■	■	■	■
2 (1.38)	2 (1.58)	70	Bx	□	■	■	□	■	■	■	■	■	■	■	■	■	■	■	■
2 (1.38)	2 (1.58)	104	Bx	■	□	□	■	■	□	□	□	■	□	□	■	■	■	■	■
2 (1.38)	1 (0.79)	73	Bx	■	■	□	■	■	■	■	■	■	■	■	■	■	■	■	■
2 (1.38)	1 (0.79)	90	Bx	■	■	□	■	■	■	■	■	■	■	□	■	■	■	■	■
1 (0.7)	2 (1.58)	8	Bx	■	■	■	■	□	■	■	■	□	■	□	■	■	■	■	■
1 (0.7)	2 (1.58)	76	Bx	■	□	□	■	■	■	■	■	■	□	■	■	□	■	■	■
1 (0.7)	2 (1.58)	94	Bx	■	■	□	■	■	■	■	□	■	■	□	■	■	■	■	■
1 (0.7)	1 (0.79)	11	Bx	■	■	■	■	■	■	□	■	■	■	□	■	■	■	■	■
1 (0.7)	1 (0.79)	13	Bx	■	■	■	■	■	■	■	□	■	■	□	■	■	■	■	■
1 (0.7)	1 (0.79)	87	Bx	□	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
1 (0.7)	1 (0.79)	91	Bx	■	■	□	■	■	■	□	■	■	■	■	■	■	■	■	■
1 (0.7)	1 (0.79)	118	Bx	■	■	□	■	■	■	■	■	■	□	■	■	■	■	■	■
1 (0.7)	1 (0.79)	377	Bx	□	□	■	□	■	■	■	■	■	□	■	■	■	■	■	■
2 (1.38)	0	15	Bx	■	■	■	■	□	□	□	■	□	□	□	■	■	■	■	■
1 (0.7)	0	19	Bx	■	■	■	■	■	□	□	□	□	□	□	■	■	■	■	■
1 (0.7)	0	28	Bx	■	■	■	■	□	■	■	■	□	■	■	■	■	■	■	■
1 (0.7)	0	75	Bx	□	■	■	□	□	■	■	■	□	■	■	■	■	■	■	■
1 (0.7)	0	159	Bx	□	■	■	□	■	■	■	■	■	■	□	■	■	■	■	■
1 (0.7)	0	200	Bx	■	■	■	■	□	■	□	□	□	□	□	■	■	■	■	■
1 (0.7)	0	342	Bx	■	□	□	■	■	■	□	□	■	■	□	■	■	■	■	■
1 (0.7)	0	363	Bx	■	□	■	■	■	■	■	■	■	□	■	■	■	■	■	■
0	1 (0.79)	12	Bx	■	■	■	■	□	■	■	■	■	□	■	■	■	■	■	■
0	1 (0.79)	36	Bx	■	■	■	■	□	■	□	□	■	■	□	■	■	■	■	■
0	2 (1.58)	43	Bx	■	■	■	■	□	□	■	□	■	□	□	■	■	■	■	■
0	2 (1.58)	68	Bx	□	■	■	□	■	■	■	■	■	□	■	■	■	■	■	■
0	1 (0.79)	72	Bx	■	□	□	■	■	□	□	□	■	□	□	■	□	■	■	■
0	1 (0.79)	81	Bx	□	■	□	□	■	■	■	■	■	■	■	■	■	■	■	■
0	1 (0.79)	151	Bx	□	■	■	■	■	■	■	■	■	■	□	■	■	■	■	■
0	1 (0.79)	188	Bx	■	■	■	■	■	□	■	□	■	□	□	■	■	■	■	■
0	2 (1.58)	293	Bx	■	□	■	■	■	□	□	□	■	□	□	■	■	■	■	■
0	1 (0.79)	440	Bx	■	■	□	■	■	■	■	■	■	■	■	□	□	■	■	■

The filled squares correspond to the presence of the KIR gene and the empty ones to the absence of the KIR gene; *ID – Genotype identification number defined according to http://www.allelefrequencies.net/kir6001a.asp, # – statistically significant

Table 3. Distribution of KIR HLA class I ligands in HBV infected patients and healthy controls.

KIR ligand	HC (n=113) n (%)	CHB (n=124) n (%)	SRI (n=20) n (%)
HLA-C1	95 (84.1)	91 (73.4)	15 (75.0)
HLA-C2	75 (66.4)	89 (71.8)	14 (70.0)
HLA-C1C1	37 (32.7)	36 (29.0)	6 (30.0)
HLA- C1C2	57 (50.4)	55 (44.4)	9 (45.0)
HLA-C2C2	19 (17.0)	33 (26.6)	5 (25.0)
HLA- B^{Bw4}	84 (74.3)	83 (66.9)	11 (55.0)
HLA-B^{w4Ile}80	72 (63.7)	62 (50.0)	6 (30.0) P=0.005; P^{corr} =0.015 OR 0.24 [0.09-0.7]
HLA-$Bw4^{Thr80}$	9 (8.0)	18 (14.5)	4 (20.0)
HLA$Bw^{4Ile80Thr8}$0	3 (2.7)	3 (2.4)	1 (5.0)
HLA- A^{Bw4}	32 (33.7)	48 (38.7)	9 (45.0)

KIR ligands

Analysis of KIR HLA C ligand frequencies showed no differences in the distribution between healthy controls and patients with HBV infection, both chronic HBV carriers and spontaneously recovered patients (Table 3).

The next step was to determine the distribution of HLA class I ligands for KIR3DL1 (alleles with Bw4 motif) in the subjects studied, but no statistically significant differences were revealed between patients and healthy controls, and between patients' subgroups as well. (Table 3).

When considering the amino acid (isoleucine or threonine) present at position 80 of the HLA-Bw4 molecule, HLA-Bw4Ile80 KIR ligands were significantly less frequent in HBV infected than controls (HBV infected patients – 47.2% vs healthy controls – 63.7%, P^{corr}=0.016). Low frequency of HLA-Bw4Ile80 positive individuals was observed in both patient subgroups (Table 3), but the difference was statistically significant for spontaneously recovered individuals. Although the lower incidence of KIR HLA-$Bw4^{Ile80}$ ligands was more pronounced in the recovered subjects (30.0%) compared to patients with chronic hepatitis B (50.0%), no significant difference was found between both patients' cohorts (P^{corr} >0.05).

KIR/ HLA ligand combinations

Frequencies of KIR/HLA class I ligand combinations were analyzed and compared between both healthy controls and patient groups, as well as between patients' subgroups (chronic HBV carriers compared to spontaneously resolved infection). Of 48 possible combinations, in which at least the receptor or ligand or both are present (inhibitory KIR/HLA class I ligand: 2DL1/ HLA-C2, 2DL2/3/ HLA-C1, 3DL1/ HLA-B^{Bw4}, 3DL1/ HLA-A^{Bw4}; activating KIR/HLA class I ligand: 2DS1/HLA-C2, 2DS2/HLA-C1, 3DS1/ HLA-B^{Bw4}, 3DS1/ HLA-A^{Bw4} and inhibitory KIR/activating KIR/HLA class I ligand: 2DL1/2DS1/ HLA-C2, 2DL2/L3/2DS2/HLA-C, 3DL1/3DS1/HLA-B^{Bw4}, 3DL1/3DS1/HLA-A^{Bw4}, data not shown), the only difference found was a tendency for higher frequency for the non-functional combination 3DL1(+)/3DS1(-)/HLA-B^{Bw4}(-) in spontaneously resolved HBV infections [30.0%; P=0.023, P^{corr}= 0.092] and chronic HBV carriers [21.0%, P=0.017, P^{corr}=0.068] compared to controls (9.7%).

DISCUSSION

The essential role of NK cells in antiviral defense is undeniable. Data on the influence of the KIR genetic background for HBV disease susceptibility, spontaneous recovery or chronicity are controversial[4-6,13,21-27].

In the present study, the role of two major genetic systems involved in the regulation of NK cell function – KIRs and KIR HLA class I ligands was investigated for the first time in hepatitis B viral infection (chronic carriers and recovered) in the Bulgarian population. The inhibitory KIR2DL5B allelic variant was found to have the lowest incidence in the subgroup of spontaneously resolved HBV infection, but the difference was significant only comparing to healthy controls and not to chronic carriers. The role of KIR2DL5 gene and its allelic variants for NK activity regulation is supported by the finding of other researchers as Shah-Hosseini et al.[16] who demonstrated a higher frequency of KIR2DL5A allele in HBV recovered individuals compared to controls. The higher distribution of inhibitory KIR3DL1*004 observed by us in chronic HBV carriers may suggest increased NK-cell inhibition in chronic disease, although some data demonstrate lack of expression of the encoded receptor[28].

In support of the hypothesis that NK-cell antiviral activity may be influenced by a genetically predetermined imbalance between activating and inhibitory KIRs, the established by us protective effect of KIR genotype consisting of six activating KIRs may be considered a mediator of predominantly activating NK-receptor signals. The complex influence of KIRs for disease susceptibility is also supported by the observed protective effect of KIR genotype composed of 4 activating KIRs and 6 inhibitory KIRs, ratio 0.67. Moreover, we found common protective KIR genotypes for HBV infection and leukemia suscepti-

bility in the Bulgarian population, supporting the suggestion that identical genetic factors may contribute to both antiviral and antileukemic NK cell activity[29].

The control of NK cell function mediated by expressed KIRs depends largely on the binding of KIRs to their appropriate ligands. There is a hierarchy in the strength of the inhibitory signal produced by KIRs binding to their respective HLA Bw4 ligands: HLA-*Bw4*^{Ile80} > HLA-*Bw4*^{Thr80} (ref.[10]). The lowest frequency of the stronger inhibitory receptor ligand group HLA-Bw4^{Ile80} in spontaneously recovered patients in the present study may indicate more effective NK antiviral defense contributing to the resolution of infection. Impact of HLA-Bw4^{Ile80} ligands on the clinical course of HBV infection was reported by other authors as well. Shah-Hosseini et al. found activating KIR3DS1+HLA-Bw4^{Ile80} combination more frequently in HBV recovered individuals than in healthy controls[17]. Moreover, the more potent HLA-Bw4^{Ile80} ligands for inhibitory KIR3DL1 were associated with an increased incidence of hepatocellular carcinoma in HBV infected patients[4].

Several research groups have reported correlations of certain KIR/HLA class I combinations with HBV infection evolution and outcome[4,6,13-17,20-22,25,27,30]. In the present study no associations with chronicity or spontaneous recovery from HBV infection according to KIR HLA class I ligand combinations were found after correction of the p value. This lack of associations to some extent contradicts the hypothesis of genetically determined activity of the immune response mediated by inhered KIR/HLA class I combination. However, a limitation of the presented study must be noted related to the smaller number of patients enrolled, especially in the group of resolved HBV infection.

CONCLUSION

There is growing evidence for associations of KIRs and HLA ligand polymorphisms with the outcome of hepatitis viral infections and the present study provides additional data to these findings. However, the exact mechanism of action of the activating and inhibitory KIRs is still unknown. This study is the first comprehensive investigation in the Bulgarian population on genes related to NK cell function – KIRs and their HLA ligands in patients with hepatitis B infection. Our initial results suggest a possible role of the KIR/HLA ligand genetic profiles for viral persistence and chronification of HBV infection. The correlation between certain KIR/HLA genotypes and effectiveness of interferon-α therapy in patients with HBV infection should also be noted and should be considered as a proof for the role of KIR on NK mediated antiviral defense[27]. In this sense the recognition of immunogenetic factors influencing innate NK responses, and associated with protection against or predisposition to chronic HBV infection may help to develop new treatment options in addition to specific antiviral therapy. The purpose of such combined strategy would be to personalize therapy, shorten the period of active infection, and reduce cases of chronicity and late complications associated with viral infections.

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