Hla-Cw7 Allele as Predictor of Favorable Therapeutic Response to Interferon-α in Patients with Chronic Hepatitis C

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Aim To evaluate the association between human leukocyte antigens (HLA) class I and therapeutic response to interferon-α in Croatian patients with chronic hepatitis C.

Methods HLA-A, -B, and -C genotyping was performed in 55 patients with sustained virological response and in 57 patients without sustained virological response to interferon-α therapy. Patients were treated in the period from 1998-2001 with interferon-α at a dose of 3 million units three times a week. Patients who became negative for hepatitis C virus RNA after 12 weeks of therapy completed 48 weeks of therapy.

Results There was no association between therapeutic outcome and frequency of HLA-A, as well as of HLA-B alleles. HLA-Cw7 was significantly more frequent in patients with than those without sustained virological response (27.0% vs 6.7%; $P = 0.011$).

Conclusion In Croatian patients with chronic hepatitis C, HLA-Cw7 is the predictor of sustained virological response to interferon-α therapy.

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Persistent hepatitis C virus (HCV) infection develops in spite of the host's multispecific humoral and cellular immunological responses that are directed toward all structural and nonstructural viral proteins. Great proportion of chronicity after HCV infection could be attributed to the ability of the virus to evade the host's immunological response (1,2). However, it is almost certain that the inability of human beings to spontaneously eliminate HCV has an inherited immunological background. Genes of the major histocompatibility complex direct the hosts' immune response. They control the expression of human leukocyte antigens (HLA), which are responsible for the presentation of processed viral antigens to the T lymphocyte receptors (3). Finally, HCV-specific cytotoxic T lymphocytes inhibit viral replication by cytokine-mediated and direct cytolytic effect (4). In contrast to acute infection, HCV-specific T cell response in established chronic infection is quantitatively weak (5). Despite inconsistencies even within the same ethnic group, different HLA class I and class II alleles have been associated with the development of chronic hepatitis, progression to liver cirrhosis and hepatocellular carcinoma, response to interferon-α therapy, vertical transmission, and extra-hepatic manifestations of HCV infection (6).

Interferon-α represents the cornerstone of chronic hepatitis C therapy. This cytokine is a multifunctional immunomodulator with a profound effect on cytokine cascade, possessing several anti-inflammatory properties (7). Nevertheless, specificities of immunomodulatory effects of interferon-α are still unknown. Interferon-α appears to achieve its antiviral effect through signal transducer and activator of transcription 3 (STAT3) signaling pathway, as well as through intracellular gene activation with inhibition of subgenomic RNA replication and suppression of viral non-structural protein synthesis (8,9). Interferon-α also increases natural killer (NK) cell activity, enhances maturation of cytotoxic T lymphocytes, and increases expression of HLA class I antigens on the cell surface, thereby promoting a more efficient elimination of infected hepatocytes through enhanced activity of cytotoxic T lymphocytes (10,11). However, the role of HLA class I immunogenetic profile in response to interferon-α therapy of chronic hepatitis C has rarely been investigated (6,12,13). The aim of this study was to investigate a possible association between HLA-A, -B, and -C genotype and the outcome of interferon-α treatment in Croatian patients with chronic hepatitis C.

Patients and methods

Study design and patients

Hundred and twenty successive patents with chronic hepatitis C were invited by a letter to participate in the study. Sixty of them were with sustained virological response and 60 patients were without sustained virological response to interferon-α therapy. Sustained virological response was defined as negative HCV RNA at the end of therapy and 6 months after the completion of therapy (14). All patients received interferon-α therapy in the period from 1998-2001 at the Department for Infectious Diseases, Split University Hospital. Inclusion criteria for participation in the study were the following: negative hepatitis B surface antigen (HBsAg), negative anti-hepatitis A IgM antibodies, negative anti-human immunodeficiency virus antibody, positive anti-HCV antibody, positive HCV RNA, elevated alanin aminotransferase level, histopathology of chronic active hepatitis, absence of other causes of liver diseases, and Croatian origin. Three patients did not meet the inclusion criteria: two of them were proven to be HBsAg positive, while the third

patient did not have the histology of chronic active hepatitis. Five patients refused to participate. Finally, 55 persons with sustained response and 57 persons without sustained response to interferon-α therapy were included in the study (Table 1).

Table 1. Principal features of patients with chronic hepatitis C according to the response to interferon-α therapy

	Sustained virological response	
Feature	yes (n = 55)	no (n = 57)
Age (years, median [range])	27 (19-47)	31 (20-53)
Female/male	12/43	9/48
Hepatitis C virus genotype:		
1	17	29
3	37	28
4	1	0
Pre-treatment viremia (IU):		
≤800 000	32	22
>800 000	23	35
Grade of fibrosis:		
0-1	17	8
2	32	28
3-4	6	21

All participants were treated with recombinant interferon-α-2a (Roferon® A, F.Hoffmann-La Roche Ltd, Basel, Switzerland) at a dose of 3 million units three times a week. Patients who became HCV RNA negative after 12 weeks of treatment completed 48 weeks of therapy. The group with sustained virological response included patients who remained HCV RNA negative 6 months after the completion of 48-week of interferon-α therapy. The group without sustained virological response included patients with no response to therapy (HCV RNA positive after 12 weeks of treatment), as well as patients with response at the end of the treatment (HCV RNA negative after 12 and 48 weeks) who relapsed after 6 months of the follow-up and became HCV RNA positive.

Anti-HCV antibodies

Anti-HCV antibodies were determined by microparticle immunoassay (15) using Abbott® SYSTEM (Abbott Laboratories, Diagnostic Division, Irving, TX, USA). The test was performed in the Central Laboratory of Split University Hospital.

HCV RNA

Serum HCV RNA was determined by Amplicor HCV test version 2.0 (Roche, Indianapolis, IN, USA) with the lowest detection level of 50 IU/mL. HCV was genotyped by INNO-LiPA II HCV (Innogenetics, Technology Park Ghent, Ghent, Belgium) using reverse hybridization method (16). Results were shown according to Simmonds classification (17). Quantification of HCV RNA was performed using Amplicor HCV MONITOR test version 2.0 (Roche, Indianapolis, IN, USA) (18). All three tests were performed in the Laboratory of Immunology, Clinic for Infectious Diseases "Dr Fran Mihaljević" in Zagreb, Croatia.

HLA-DNA

DNA was isolated using QIAmp® DNA Blood Midi Kit (QUIAGEN GmBH, Hilden, Germany) from a sample of 2 mL heparinized full blood. HLA typing was performed on Auto-LIPA (Abbot GmBH Diagnostica, Delkenheim, Germany) instrument, using the technique of reverse hybridization (19). The test was carried out in the Laboratory for Tissue Typing, Department for Clinical Pathophysiology, Split University Hospital. The frequencies of HLA-A, -B, and -Cw alleles in healthy Croatian population were previously published (20,21).

Liver biopsy

Samples of the liver were obtained by blind needle biopsy. Histological activity index (HAI = 0-22) was determined according to Kondell et al (22). Stage of fibrosis (F) was assessed by METVIR scoring system (F0 – no fibrosis, F1 – portal fibrosis without septa, F2 – portal fibrosis with rare septa, F3 – numerous septa without cirrhosis, F4 – cirrhosis) (23).

Ethical standards

Ethical norms, the Croatian legislation, and international conventions were respected. Ethical approval was provided by the Ethics Committee of Split University Hospital, and patients gave the informed consent.

Statistical analysis

Differences between the variables were analyzed by χ^2 test and $P<0.05$ was considered statistically significant. Data analysis was performed using the Statistical Package for the Social Sciences for Windows, version 13 (SPSS Inc., Chicago, IL; USA).

Results

Groups of patients with and without sustained virological response were similar according to age, sex, HCV genotype, pretreatment viremia, and grade of liver fibrosis (Table 1).

Frequencies of HLA-A did not differ significantly between the groups ($\chi^2 = 10.13$; $P = 0.518$) (Table 2). Homozygosity for HLA-A was found in 3 examinees with sustained virological response (2 with A1/A1 and 1 with A2/A2) and 9 examinees without sustained virological response (1 with A1/A1, 4 with A2/A2, 1 with A3/A3, 2 with A24/A24, and 1 with A31/A31).

Table 2. Distribution of human leukocyte antigens-A (HLA-A) in patients with chronic hepatitis C according to the response to interferon-α therapy

	Sustained virological response in patients with a given allele	
HLA	yes (n = 55)	no (n = 57)
A 1	11	13
A 2	24	28
A 3	8	13
A 11	8	5
A 23	4	1
A 24	19	16
A 25	3	5
A 26	11	8
A 29	1	0
A 30	2	2
A 31	3	7
A 32	8	3
A 33	2	5
A 66	1	0
A 68	4	7
A 74	1	1

Table 3. Distribution of human leukocyte antigen-B (HLA-B) in patients with chronic hepatitis C according to the response to interferon-α therapy

	Sustained virological response in patients with a given allele	
HLA	yes (n = 55)	no (n = 57)
B 6	1	0
B 7	7	4
B 8	6	2
B 13	2	2
B 14	2	3
B 15	8	6
B 16	2	0
B 18	12	8
B 27	4	6
B 35	14	9
B 36	0	1
B 37	1	2
B 38	5	8
B 39	3	2
B 40	3	3
B 41	1	2
B 44	8	8
B 49	2	3
B 50	2	0
B 51	8	15
B 52	1	0
B 55	1	0
B 56	1	1
B 57	3	1
B 58	0	2
B 59	0	2
B 60	2	0
B 61	1	1
B 62	0	2
B 67	1	2
B 75	1	1

Frequency of HLA-B did not differ significantly between the groups ($\chi^2 = 8.40$; $P = 0.395$) (Table 3). Determination of HLA-B failed in 4 patients with sustained virological response, as well as in 9 patients without sustained response to interferon-α therapy. HLA-B8/B8 homozygosity was found in a single patient without sustained virological response.

Frequency of HLA-Cw7 allele in patients with sustained virological response significantly exceeded that in patients without sustained virological response to interferon-α therapy (Table 4). Determination of HLA-Cw failed in 5 patients with sustained virological response and in 5 patients without sustained virological response. HLA-Cw3/Cw3 homo-

Table 4. Distribution of human leukocyte antigen-Cw (HLA-Cw) in patients with chronic hepatitis C according to their response to interferon-α therapy

	Sustained virological response in patients with a given allele	
HLA	yes (n = 55)	no (n = 57)
Cw 1	6	13
Cw 2	9	11
Cw 3	8	12
Cw 4	8	5
Cw 5	9	6
Cw 6	5	10
Cw 7	27*	7*
Cw 8	3	6
Cw 9	0	2
Cw 11	0	1
Cw 12	15	10
Cw 14	2	3
Cw 15	2	6
Cw 16	3	6
Cw 17	2	3
Cw 19	1	0
Cw 20	0	1
Cw 23	0	1
Cw 25	0	1

*Significant difference between patients with and without sustained virological response (χ^2 test = 19.78; P = 0.011).

zygosity was found in one patient without sustained virological response.

Discussion

Our study showed an association between HLA-Cw7 alleles and sustained virological response to interferon-α therapy, suggesting that patients with HLA-Cw7 allele clear HCV-infected hepatocytes more efficiently. HLA-Cw7/Cw7 homozygosity was not found, while the association between the outcome of interferon-α therapy and alleles that were found in our patients with homozygosity (HLA-A1/A1, -A2/A2, -A3/A3, -A24/A24, -A31/A31, -B8/B8, and -Cw-3/Cw3) was not demonstrated. Although certain types of HLA homozygosity were shown to be associated with the persistence of HCV infection (24), progression of liver fibrosis (25), and vertical transmission of HCV infection (26), we were not able to find reports about the association with interferon-α treatment response. A small number of studies, with conflicting results, investigated the association of HLA-A, -B, and -C alleles and interferon-α therapy. Miyaguchi et al (12) found that in Japanese patients with chronic hepatitis C HLA-B55, -B62, -Cw3, and -Cw4 alleles were associated with better response to interferon-α, as well as with lower pre-treatment viral load. Therefore, their results do not allow us to conclude that a favorable therapeutic outcome is a result of direct mutual influence of HLA and interferon-α. As opposed to the results from both Japanese and our study, Romero-Gomez et al (13) did not find the association between HLA-A,-B, and -C alleles and interferon-α monotherapy. Although certain HLA-A, -B, and -C genotypes could be associated with the outcome of interferon-α therapy, this merely does not appear to be of crucial influence on the therapeutic outcome. Moreover, one cannot describe the exact mechanism of this influence. Shiina et al (27) suggested the possibility that the favorable effect of interferon-α in patients with chronic hepatitis C may not include the induction of cytotoxic T lymphocyte. In addition, Freni et al (28) suggested that during a successful treatment of chronic hepatitis C with interferon-α, the clearing of virus occurred due to a direct antiviral, rather than immunologically mediated mechanism. Finally, several HLA class II alleles appear to significantly influence interferon-α treatment response (6).

Interferon-α increases NK cell activity and HLA-Cw7 belongs to group 1 ligands of killer immunoglobuline-like receptors (KIR) that are critical regulators of NK cell activity (29). NK cells are the first line of innate immunity and their activity can affect the course of acute HCV infection (30). Therefore, there is also a possibility that, in Croatian patients, interferon-α acts through HLA-Cw7 that binds certain genotypes of KIRs. However, the importance of NK cell activity during interferon-α therapy of chronic hepatitis C requires further investigations. Therefore, it is very probable that, in majority of patients, interferon-α

achieves therapeutic effect by the combination of its immunomodulatory and direct antiviral performances.

The significance of our results is limited by relatively small number of examinees. Association between HLA-A,-B, and -C genotype and therapeutic response to interferon-α in Caucasian patients with chronic hepatitis C was rarely investigated. Apart from our work, it was only the case in the Spanish study, although being a Caucasian was not an inclusion criterion in their investigation (13).

In conclusion, determination of HLA-Cw7 in Croatian patients with chronic hepatitis C may serve as a prognostic factor of therapeutic response to interferon-α. A better understanding of this issue requires more thorough knowledge of relevant mechanisms of action of interferon-α, as well as further investigation into its association with HLA-A,-B, and -C genotype in a larger number of examinees with chronic hepatitis C.

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