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Role of viral and host factors in the pathogenesis of Hepatitis C Virus infection and in the response to interferon treatment

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Abstract

Background and purpose: *To asses the role of viral and host factors in the pathogenesis of Hepatitis C virus (HCV) infection, serum HCV-RNA, immunological and HLA studies were performed in patients with chronic hepatitis C and symptomfree, HCV-positive blood donors with normal alanine aminotransferase (ALT) Materials and methods: Virological (anti-HCV and quantitative serum HCV-RNA bDNA test, HBsAg, anti-HBc), and immunological (serum immunoglobulin, autoantibodies, immune complex, cryoglobulin, lymphocyte subset, lymphoproliferative response, natural killer cell activity and HLA A, B, C, DR, DQ) studies were done.*

Results: *serum HCV-RNA levels were significantly lower in symptomfree HCV-positive individuals, than in chronic hepatitis C patients. Good responders to interferon (IFN) showed also lower HCV-RNA load than non-responder patients treated with IFN. Previous HBV infection occurred only in chronic hepatitis patients but not symptomfree individuals. IFN non-responders had more frequent HBV+HCV coinfection than responders. Various autoantibodies have been found mostly in patients, who had higher serum immunlobulin levels, while lymphocyte count was higher in symptomfree cases. NK cell activity significantly decreased in HCV patients. HLA DR3 and DRQ2 antigens occurred with higher frequency in patients with chronic hepatitis C as compared with normal control. Non-*

Abbreviations:

ALT	- alanine aminotransferase
ANA	- antinuclear antibody
anti-HBc	- hepattis B virus core antibody
AST	- alanine aspartate transaminase
bDNA	- branched deoxiribonucleic acid
HBsAg	- hepatitis B surface antigen
HBV	- hepatitis B virus
HCV	- hepatitis C virus
HCV-RNA	- hepatitis C virus ribonucleic acid
HLA	- human leukocyte antigen
IFN	- interferon
ITP	- immune thrombocytopenia
LKM	- liver kidney microsomal antibody
LSP	- liver specific protein
NK cell	- natural killer cell
PCA	- parietal cell antibody
RF	- rheumatoid factor
SMA	- smooth muscle antibody
SS-A	- Sjögren's syndrome associated antibody
Thy	- thyreoid microsomal antibody

responders to IFN showed higher HLA DR3 occurrence than responders. Prevalence of HLA DR5 positivity was lower in chronic HCV infection as compared with healthy controls.

Conclusion: *serum HCV-RNA level as viral factor, and HLA as host factor may determine the course of HCV infection and the response to antiviral treatment. HLA DR3 functions as a marker of susceptibility and a negative predictor of therapeutic response, while HLA DR5 may have protective effect in HCV infection.*

INTRODUCTION

Hepatitis C virus (HCV) infection is characterized by a high rate (60-80%) chronic carrier state, associated with persisting viral replication that results in various forms of liver injury, ranging from mild, symptomfree acute or chronic hepatitis over severe active chronic hepatitis or cirrhosis to hepatocellular carcinoma *(1, 3, 16)*. In addition, since HCV infects not only hepatocytes but monocytes-macrophages, lymphocytes, salivary and lacrimal cells, HCV can cause extrahepatic manifestations too, such as cryoglobulinemia, vasculitis, arthritis, nephritis, Sjögren's syndrome, thyreoiditis, ITP. Thus, HCV infection can be regarded as a systemic disorder *(5, 14)*.

Pathogenetic mechanisms of HCV-related tissue damage are not clear elucidated, but importance of viral and host factors is presumed. It became evident, that HCV 1b genotype is frequently resistant to antiviral (interferon) treatment *(3, 4, 17)*. On the other hand, the outcome of HCV infection may depend on the age, gender and genetically determined immune response of the host. In addition, some environmental factors, such as previous hepatitis B virus infection (HBV) or alcoholism also may influence the progression of HCV-related disease *(1, 2, 16)*.

In the recent study we wanted to assess the role of HCV-RNA level as viral factor, and immunological and HLA markers as host factors in the course of HCV infection and in the response to antviral (interferon) treatment. Comparative studies have been performed in symptomfree „healthy" HCV-positive blood donors (with normal alanine aminotransferase) and patients with chronic hepatitis C. Furthermore, two subgroups of HCV patients treated with interferon, namely good responders and non-responders were also compared.

PATIENTS AND METHODS

Patients

Forty eight (23 males and 25 females) patients with chronic hepatitis C were studied. Their mean age was 44.6±12.0 years (range 20 to 64 years), mean alanine amino-transferase (ALT) level was 179±101 IU (90 to 500 IU). Out of them 33 patients have been treated with interferon-alpha 2b at a dose of 3-5 MU three times a week, when interferon treatment resulted in sustained remission in 11 patients, while 22 patients were non-responders.

Thirty six (13 males, 23 females) symptomfree, HCV-positive individuals with repeatedly normal ALT values were also investigated, their mean age was 35 ± 10 years (21 to 45 years).

Three hundred and forty healthy blood donors served as normal controls.

Methods

Biochemistry. Serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phospghatase, (ALP), gammaglutamyl transpeptidase (GGT), total protein, albumin, prothrombin, serum iron, ferritin, creatinine were measured.

Histology. Liver biopsy speciments of chronic hepatitis C patients were studied based on standard criteria and scored for histological activity and fibrosis according to a numerical scoring system. Symptomfree HCV-positive indiviuals with repeatedly normal ALT activity were not biopsied.

Virology. Tests for hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), anti-HCV, were performed using ELISA test systems (ABBOTT) as recommended by manufacturers.

Detection of HCV-RNA was performed by polymerase chain reaction (PCR) using AMPLICOR (Roche) test, while for quantitative measurements bDNA technique (Chiron) was applied.

Immunology

Humoral immunity. Serum immunoglobulins (IgA, IgG, IgM), autoantibodies: antinuclear (ANA), anti-DNA, antimitochondrial (AMA) smooth muscle (SMA) liver cell mebrane (LMA), liver-kidney microsomal (LKM), parietal cell (PCA), thyreoidea microsomal, anti-SSA antibodies, circulating immune complexes, cryoglobulins, rheumatoid factors were detected by standard immunochemical, or indirect immunocytochemical or ELISA techniques.

Cellular immunity. Blood picture, absolute granulocyta and lymphocyte counts, total B and T cell, CD4+, CD8+, CD16+ cell counts were analysed using fluorescent flow cytometry. Mitogen induced lymphoproliferative response to phytohemagglutinin as well as natural killer cell (NK cell) activity (single cell method on K562 target cells) was measured.

HLA A,B, C, DR, DQ determinatons were carried out by serological method using standardized microlymphocytotoxicity test according to the National Intitute of Health.

Statistical analysis: Student's t-test and X-square test were used.

RESULTS

Viral studies

Serum HCV-RNA load: in symptomfree anti-HCV-positive individuals with normal ALT, serum HCV-RNA level was significantly lower as compared with chronic hepatitis C patients: 1.886 ± 2.397 MEQ/ml vs 4.620±5.500 MEQ/ml. ($p < 0.05$). Similarly, in those patients who responded well to interferon treatment, serum HCV-RNA load was lower than in non-responders (1.433±2.581 vs 6.610±3.400, $p < 0.05$).

Previous (or ongoing) **HBV infection** (HBsAg or anti-HBC positivity) did not occur in symptomfree HCV-positive donors, while in patients with chronic hepatitis C, HBsAg carriership occurred in 11%, and anti-HBc positivity in 35%, respectively.

Immunological findings

Humoral immunity. Mean values of serum immunoglobulin levels are shown in Table 1. In symptomfree HCV- positive individuals immunglobulin values scarcely deviated from the normal control levels, while in chronic hepatitis C patients all classes of immunoglobulins significantly increased. Concerning the prevalence of auto-antibodies, cryoglobulins and immune complexes, there was also a difference between „healthy" carriers and patients: in the latter group all of these, but anti-thyreoid antibodies occurred with higher frequency.

TABLE 1

Humoral immune parameters.

	Serum immunoglobulins (g/l)		
	IgA	IgG	IgM
normal control (n=50)	2.13 ± 0.6	12.25 ± 2.00	1.37 ± 0.42
HCV-donors: (n=13)	2.05 ± 0.59	15.65 ± 3.65	1.80 ± 0.82
HCV-hepatitis (n=36)	3.09 ± 1.60	18.40 ± 4.42	2.77 ± 1.71

	Prevalence of autoantibodies							
	anti-LSP	Rf	LKM	ANA	SMA	PCA	Thyab	SS-A
normal control (n=40)	7.5%	10%	2%	5%	5%	7.5%	7.5%	5.%
HCV-donors: (n=36)	n.d.	22%	n.d	6%	0	3%	13%	8%
HCV-hepatitis : (n=36)	80%	42%	22%	11%	22%	22%	0%	20%

	Prevalence of cryoglobulin and immune complex	
	cryoglobulin	immune complex
normal control (n=40)	5%	10%
HCV-donors: (n=36)	5%	16%
HCV-hepatitis: (n=36)	44%	33%

Cellular immune parameters

In asymptomatic HCV carriers total T cell, CD4+ and CD8+ T cell counts as well as CD4/CD8 ratio significantly increased as compared with normal controls, while the increase in B cell count and decrease in NK cell count was not significant.

In patients with hepatitis C, no significant difference was noted in lymphoid cell counts relative to the normal. Mitogen (PHA)- induced lymphoproliferative response showed a moderate but not significant decrease, however NK cell activity was significantly lower in HCV-positive patients (Table 2)

HLA studies

A significant increase in the prevalence of HLA B8, HLA DR3, and HLA DQ2 alleles was found in patients with chronic hepatitis C but not in HCV carriers, while HLA DR5 allele occurred with lower frequency in both HCV-positive groups as compared with the normal control (Table 3).

Predictors of response to interferon

When analysing the features of patients who responded to interferon treatement with sustained remission, and those who were non-responders, some significant differences were noted. (Table 4) Female sex, shorter time elapsed from transfusion, lower serum HCV-RNA level, lower serum ferritin were found as predictors of response, while presence of fibrosis, previous HBV infection, and HLA B8, DR3, DQ2 , or HLA DR3 alleles, frequently occurring in non-responders, seemed to be as "negative predictors".

DISCUSSION

Our results suggest that following HCV infection, a high serum HCV-RNA level may predispose not only for

TABLE 2

Cellular immunity studies.

	Lymphocyte subsets (cell/ul)				
	CD4+T	CD8+T	CD4/CD8	NK	B-cell
normal control (n=40)	757±350	447±314	1.9±0.7	360±217	192±92
HCV-donors (n=16)	1195±532	954±276	1.1±0.2	317±163	275±174
HCV-hepatitis (n=32)	711± 268	432±181	1.7±0.6	276±157	183±144

	Lymphoproliferative response	NK-cell activity
	(PHA stimulation)	(S.I.) (%)
normal control (n=40)	122±52	3.44±1.10
HCV-donors (n=12)	111±68	n.d
HCV-hepatitis (n=32)	103±53	2.06±1.67

chronicity of liver disease but for a poor response to interferon therapy. Symptomfree HCV-positive individuals showed lower HCV-RNA serum level than patients with chronic hepatitis C, as well as good responders to interferon have also lower HCV-RNA load than no-responders. Although others have also described similar findings, there are contradictory reports as well regarding the importance of this factor in the pathogenesis of chronic hepatitis C *(1, 10)*. Further significant virological factors are genotypes of HCV and heterogeneity of the virus, that is the number of quasi-species that developed during chronic infection *(3, 4)*. Until now we were not able to investigate these latter parameters, but the first studies in Hungary by Héjjas and Gervain have suggested, that about 85% of HCV-positive individuals were infected with HCV1 genotype in our country (personal communication). It is well known, that HCV 1b genotype frequently results an interferon-resistant chronic hepatitis C *(1-4)*.

TABLE 3

*Prevalence of **HLA antigens**.*

	HLA A1B8	B8	DR3	DR4	DR5	DQ2
normal control	36/340 (10.6%)	52/340 (15.3%)	16/77 (20.7%)	17/77 (22.0%)	30/77 (39.0%)	14/77 (18.2%)
HCV-pos-ve donors	4/30 (13.3%)	8/30 (26.6%)	14/30 (46.6%)	4/30 (13.3%)	4/30* (13.3%)	10/30 (33.3%)
HCV-pos-ve hepatitis	9/36 (25.0%)	13/36* (36.1%)	14/25* (56.0%)	4/25 (16.0%)	4/25* (16.0%)	9/25 (36.0%)

*p<0.05

Concerning the host's side, our recent findings, in accordance with others *(5, 8)* and with our previous studies *(12, 13)* show an immune response of TH2 dominance and B cell activation by HCV, that is manifested in hypergamma-globulinaemia, autoantibody- and cryoglobulin production, accompanied with a moderate decrease in mitogen-induced lymphoproliferative response and a significant decrease in NK cell activity. NK cell activity has rarely been studied in hepatitis C, but was also found to be diminished *(6)*. Symptomfree HCV carriers showed an elevation in peripheral blood T cell and B cell count with a decrease in CD4/CD8 ratio, as it was described in our original report *(13)*.

The high prevalence of HLA B8, HLA DR3 alleles found in HCV-infected patients suggests that these genetic markers - known as predisposing background for autoimmunity (and for defective suppressor activity) *(9)* - may play a role even in the development of chronic hepatitis and of B cell hyperactivity related to HCV infection.

On the other hand, HLA DR5 phenotype, that occurs with lower frequency in HCV carriers, may have a "protective" effect against chronicity of HCV infection. Peano et al in HLA DR5 positive patients have also found HCV positive liver disease with lower frequency, than in HLA DR5 positive ones, supporting our findings *(15)*.

When treating HCV patients with interferon, HLA DR3 can be regarded as a "negative predictor", since this allel occurred more frequently in non-responders. Concerning other predictors of response to interferon, among our good responders there were more females, the time elapsed from the transfusion was shorter, the number of patients with double (HBC+HCV) infection was smaller, serum ferritin level was significantly lower and fibrosis was more rare.

TABLE 4

Predictors of response to interferon.

Predictors	Good responders (sustained remission) n=11	No-responders n=22
Gender (male/female)	2/9	13/9
Time elapsed from transfusion (months)	30.0±32.1	186.6±202.0
Serum ferritin (ug/l)	36.7±20.0	176.4±212.0
Serum HCV-RNA (MEQ/ml)	1.433±2.581	6.610±3.400
HBV infection (HBsAg/anti-HBc pos)	1/11 (10%)	10/17 (59%)
Fibrosis prevalence	2/11 (18%)	18/22 (82%)
HLA DR3	4/9 (44%)	8/9 (89%)
HLAB8,DR3, DQ2	1/9 (11%)	6/9 (67%)

Others' data made it clear, that hepatic iron concentration could influence the outcome of HCV infection, since hepatic iron as well as serum ferritin level was found to be higher in non-responders compared with responders. In such cases iron removal by phlebotomy seemed to be a useful therapeutic modality in combination with interferon *(11, 17, 18)*.

In conclusion, both viral and host factors are of importance in the course of HCV infection, and all of these influence also the response to antiviral treatment.

We think, that in patients with "negative predictors", that is e.g. males with long lasting (longer than 2-3-year duration) disease, with high HCV-RNA level, or double (HBV+HCV) infection, or with fibrosis in liver histology, the result of usual monotherapy will be suboptimal. In such cases, a more aggressive treatment schedule should be necessary, e.g high dose induction therapy with interferon or its combination with other antivirals (ribavirin, amantadine or, in the next future, protease inhibitors).

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