

Viral clearance or persistence after acute hepatitis C infection: interim results from a prospective study

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Key words: acute hepatitis; hepatitis C virus; immune response; T cells; viral clearance.

Summary. *Objective.* Hepatitis C virus infection (HCV) has a high rate of chronic evolution; however, the underlying mechanisms remain to be elucidated. We investigated natural clinical, virological, and immunological course of acute HCV infection in order to identify possible prognostic factors of spontaneous resolution and to gain more understanding of early characteristics responsible for viral clearance or persistence.

Materials and methods. Eight patients with acute symptomatic hepatitis C were prospectively followed up for more than 6 months (range, 8–14 months). None of the individuals received antiviral therapy during the study period. We analyzed biochemical, virological, and immunological parameters of these patients detected at different time-points of the follow-up. Plasma HCV RNA was quantitated using TaqMan® real-time polymerase chain reaction. Virus-specific CD4⁺ T cells were enumerated by interferon-gamma (IFN-γ) ELISpot assay.

Results. Two of eight individuals resolved HCV spontaneously, while the remaining patients developed chronic HCV infection. HCV RNA became undetectable within 14 days of the study, followed by a rapid alanine aminotransferase normalization in patients with resolved infection. On the contrary, chronically infected subjects demonstrated persistent viremia or intermittently undetectable HCV-RNA, accompanied by polyphasic alanine aminotransferase profile throughout the study. Patients with self-limited hepatitis C displayed the strongest virus-specific CD4⁺ T (IFN-γ) cell reactivity within the first weeks of the follow-up, while persistently infected subjects initially showed a weak antiviral CD4⁺ T (IFN-γ) cell response.

Conclusions. In most cases, acute hepatitis C progresses to chronic disease. Viral clearance within the first month after clinical presentation accompanied by monophasic alanine aminotransferase profile could predict recovery. Early and strong CD4⁺/Th1 immune response against HCV might play an important role in the disease resolution.

Introduction

More than 170 million people worldwide are infected with the hepatitis C virus (HCV) (1, 2). HCV infection is characterized by a high rate of chronic evolution. Nearly 90% of newly infected patients develop chronic hepatitis (3, 4). Hence, at present HCV infection is the leading cause of liver cirrhosis and hepatocellular carcinoma in the majority of Western countries (5, 6). Unfortunately, the mechanisms responsible for the viral clearance or persistence remain unclear.

Cellular immune responses are thought to play an important role in the immunopathogenesis of HCV infection (7–13). Viral clearance is associated with vigorous and permanently maintained T cell responses

that target multiple HCV epitopes during acute infection. By contrast, patients developing chronic hepatitis C appear to display weak, narrowly focused, and often dysfunctional antiviral cell-mediated immunity. Early interactions between the virus and the host immune response seem to be critical in determining outcome of the disease. In this respect, patients with acute HCV infection provide an ideal opportunity to understand the correlates for successful or failed immune response to hepatitis C virus.

The problem is that acute hepatitis C is often clinically mild or completely asymptomatic and is rarely recognized outside prospective surveillance after exposure to known risk factors (14, 15). Thus today, predominantly symptomatic cases are diagnosed,

which comprise approximately one-third of acute HCV patients (2, 16). In addition, the epidemiology of the disease has changed during the past decade, and the incidence of new infections has decreased in most of the developed countries with a progressing reduction of transfusion-associated forms (6), making larger studies increasingly difficult. Accordingly, there are still a number of important unresolved issues concerning the treatment of acute hepatitis C (17, 18). It is not clear yet which patients should be treated, when therapy should be started, and what regimen is optimal. Both from the clinical and research perspectives, it is crucial to recognize acute hepatitis C and to understand its clinical, virological, and immunological aspects, which are essential for the development of more effective treatment and prevention strategies.

The present study is a part of a larger prospective project for immunological determinants of HCV clearance or persistence in patients with acute hepatitis C. In this article, we demonstrate clinical and virological course, also frequency of antiviral CD4⁺ T (IFN- γ) cells detected in a group of patients with self-limited or chronically evolving acute symptomatic hepatitis C virus infection. We investigated possible predictors of spontaneous resolution as well as immunological factors responsible for viral clearance or persistence.

Materials and methods

Patients and samples

Eight patients with acute hepatitis C were enrolled at the National University Hospital of Tuberculosis and Infectious Diseases in Vilnius (Lithuania) between January 2004 and August 2004. Diagnosis of acute hepatitis C was based on high levels of serum alanine aminotransferase (ALT) at least 10 times the upper limit of normal, anti-HCV positivity at the time of the diagnosis (two patients had documented negative results of anti-HCV tests in the previous 9–15 months), detection of HCV RNA, known or suspected exposure to HCV within past four months, exclusion of HBV (hepatitis B virus) and HIV (human immunodeficiency virus) infections, autoimmunity, alcohol abuse, toxins, and metabolic factors.

Patients were investigated longitudinally for one year with the following visits: at enrollment (baseline) and then at weeks 1, 2, 3, 4, 8, 12, 16, 24, 32, 40, and 48 during the study. At each visit, blood was obtained for biochemical, virological, and immunological assessment. Samples of peripheral blood mononuclear cells (PBMCs), serum, and plasma were cryopreserved

and stored at -80°C in the laboratory of Vilnius University for the subsequent shipment and analysis in London. All observations reported in this study refer to the natural course of HCV infection, that is, in the absence of antiviral therapy.

All patients gave informed consent before entering the study. The protocol of the study was approved by the Lithuanian Bioethics Committee.

Biochemistry tests

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and albumin measurements in serum were performed using standard assays in the Laboratory Diagnostics Centre of Vilnius University Hospital, Santariškės Clinics.

Anti-HCV antibody testing

HCV antibodies were determined using a third-generation enzyme-linked immunosorbent assay (ORTHO HCV 3.0 ELISA Test System with Enhanced SAVE, Ortho-Clinical Diagnostics, USA) according to the manufacturer's instructions.

Detection of HCV RNA

Diagnosis of HCV infection was confirmed by determining HCV RNA using a qualitative PCR-based assay with a sensitivity of 50 IU/mL (AMPLICOR Hepatitis C Virus Test, version 2.0, Roche, USA) according to the manufacturer's instructions.

Levels of circulating HCV RNA were measured by quantitative assay at all time-points of the follow-up. Total RNA was extracted from 140 μL of thawed plasma using QIAamp Viral RNA Mini Kit (Qiagen, Crawley, UK). HCV RNA was quantitated by a real-time reverse-transcription polymerase chain reaction (RT-PCR). Briefly, HCV RNA was reverse transcribed and amplified in a single-tube one-step process using QuantiTect RT-PCR (Qiagen) and quantitated with the TaqMan[®] fluorogenic detection system (ABI Prism 7700; Applied Biosystems, Warrington, UK). Thermal cycling parameters were as follows: 50°C for 30 minutes (reverse transcription), 95°C for 5 minutes (Taq DNA activation and reverse transcriptase inactivation). PCR amplification consisted of 45 cycles of 94°C for 15 seconds (denaturation) and 60°C for 60 seconds (annealing/extension). All samples were tested in duplicate, and the quantitative values were determined from an internal standard, which had been validated according to the WHO International Standard for HCV [96/790] (National Institute for Biological Standards and Controls, Potters Bar, UK).

HCV genotyping

HCV genotypes were determined using a line probe assay (VERSANT HCV Genotype Assay (LIPA), Bayer Healthcare, Belgium) according to the manufacturer's instructions.

Isolation of peripheral blood mononuclear cells (PBMC)

Total PBMCs were isolated from 40 mL heparinized whole blood via density gradient centrifugation over Lymphoprep (Axis-Shield, Oslo, Norway). Separated PBMCs were cryopreserved in media containing fetal calf serum (FCS) (Biological Industries, Israel), dimethyl sulfoxide (DMSO) (Sigma-Aldrich, UK), and RPMI 1640 medium (Biological Industries), using an isopropanol container. Cryopreserved PBMCs were stored at -80°C and thawed immediately before use. The viability of the PBMCs was $\geq 90\%$.

Enumeration of CD4⁺ HCV-specific IFN- γ -producing cells

HCV-specific CD4⁺ T (IFN- γ) cells were detected using enzyme-linked immunospot (ELISpot) assay. The assay was performed with duplicate cultures of 2×10^5 PBMCs. PBMCs were tested using recombinant HCV proteins (Microgen GmbH, Neuried, Germany) – core, NS3, NS4, and NS5 – at $1 \mu\text{g/mL}$. Positive control antigen was $1 \mu\text{g/mL}$ phytohemagglutinin (PHA) (Sigma, Dorset, UK). Medium alone was used as a negative control: RPMI 1640 medium (Gibco, UK), supplemented with sodium hydroxide (BDH Laboratory Supplies, Poole, England), HEPES buffer, L-glutamine, penicillin/streptomycin (Gibco), and 10% human AB serum (JR Scientific, Woodland, CA). PBMCs were preincubated with HCV and control antigens for 20 h at $37^{\circ}\text{C}/5\% \text{ CO}_2$ in 96-well round-bottom plates. In parallel, 96-well ELISpot plates (BD Biosciences, San Diego, USA) were coated with $100 \mu\text{L}$ of $5 \mu\text{g/mL}$ anti-IFN- γ (BD Biosciences) for 16 h at 4°C , washed and blocked with RPMI/10% human AB serum. The preincubated PBMCs then were transferred to the antibody-coated plates and incubated for 24 h at 37°C , $5\% \text{ CO}_2$. Following this incubation, IFN- γ -producing cells were detected according to manufacturer's (BD Biosciences) instructions. After plates were air-dried overnight at room temperature, spots were counted with the AID ELISpot reader system (AID Diagnostika, GmbH, Strassberg, Germany). The number of specific spot-forming cells was determined as the mean number of spots in the presence of an antigen minus the mean number of spots in the wells with medium only and expressed as per million

PBMCs. Depletion of CD4⁺ T cells using immunomagnetic beads abrogated a positive result by the ELISpot assay, thus confirming CD4⁺ T cell reactivity to HCV antigens.

Statistical analysis

Differences of quantitative data between two groups of patients were assessed by Mann-Whitney *U* test. Correlation between continuous parameters was determined by Spearman's rank correlation coefficient (r_s). Categorical variables were analyzed using Fisher's exact test. A *P* value of less than 0.05 was considered significant. Statistical analysis was performed with the SPSS 11.0 program.

Results

Patient characteristics

Eight patients diagnosed with acute HCV infection were included in the study. The median time between the first clinical symptoms and enrollment to the study was two weeks (mean \pm standard deviation (SD), 15 ± 3.2 days; range, 11–20 days). Baseline characteristics of the individuals are summarized in Table. The group included four women and four men, aged 20 to 50 years (median age, 28 years; mean age, 33 ± 13.1 years). The most common risk factors for acute hepatitis C were medical procedures ($n=3$) and sexual exposure ($n=2$). The rest identified sources of infection were injecting drug use ($n=1$) and razor sharing with an HCV-positive family member ($n=1$). HCV genotypes 1 and 3 were predominant among acutely infected individuals (1a, $n=2$; 1b, $n=2$; 2c, $n=1$; 3a, $n=3$). All patients at presentation were symptomatic with elevated serum bilirubin levels (at enrollment median bilirubin level, $48.5 \mu\text{mol/L}$; mean, $70.9 \pm 48.3 \mu\text{mol/L}$; range, 25.1 – $143.2 \mu\text{mol/L}$) and jaundice. Other symptoms included weakness ($n=6$), pain in the upper right quadrant of the abdomen ($n=5$), poor appetite ($n=4$), nausea ($n=2$), dizziness ($n=2$), slight fatigue ($n=1$), itching ($n=1$), vomiting ($n=1$), and diarrhea ($n=1$). At baseline, the median ALT level of acute patients was 757 U/L (mean ALT, $883 \pm 540 \text{ U/L}$; range, 215–1710 U/L). All subjects were viremic at the beginning of the study. Viral load ranged from $3.99 \log_{10}$ copies/mL to $7.67 \log_{10}$ copies/mL (median viral load, $6.6 \log_{10}$ copies/mL; mean, $6.2 \pm 1.26 \log_{10}$ copies/mL) at the time of enrollment. Seven patients (#1, #3–#8) were anti-HCV positive at the onset of acute hepatitis C. Two of them (patients #4 and #7) had documented negative results of anti-HCV tests in the previous 9–15 months. HCV-specific antibodies were not detected in the remaining patient #2 at baseline and the end of

Table. Baseline characteristics of patients with acute hepatitis C virus infection

Patient No.	Sex	Age (years)	Source of infection	Jaundice	HCV genotype	Outcome	ALT (U/L)	AST (U/L)	Total bilirubin ($\mu\text{mol/L}$)	HCV RNA (\log_{10} copies/mL)
1	F	47	Sexual exposure	Yes	3a	Self-limited	800	569	120.8	6.50
2	F	20	Razor sharing	Yes	3a	Self-limited	1710	980	34.9	6.75
3	M	23	Drugs	Yes	3a	Chronic	535	76	25.1	3.99
4	M	21	Sexual exposure	Yes	1a	Chronic	372	53	27.7	4.46
5	F	33	Medical procedure*	Yes	1b	Chronic	1497	966	118.4	7.67
6	F	50	Medical procedure**	Yes	1b	Chronic	1219	837	40.7	6.72
7	M	23	Medical procedure*	Yes	1a	Chronic	713	317	56.3	6.52
8	M	47	Unknown	Yes	2c	Chronic	215	249	143.2	6.71

No statistically significant difference between self-limited and chronic patients was found for the indicated parameters.

Normal range of biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST) (male ≤ 40 U/L, female ≤ 35 U/L); total bilirubin $< 17 \mu\text{mol/L}$.

F – female; M – male; *dental treatment; **vein surgery.

the follow-up. None of the individuals received antiviral therapy during the study period. All subjects were negative for HIV-1 and HIV-2 antibodies as well as for HBV surface antigen at baseline.

Clinical course and outcome of acute HCV infection

Clinical outcome of patients with acute hepatitis C was determined after a follow-up of more than 6 months (range, 8–14 months). Patient #1 and patient #2 lost HCV RNA within the first two weeks of the study, followed by bilirubin and ALT normalization. Liver enzymes remained normal and HCV RNA undetectable in these subjects until the end of the follow-up (Fig. 1, upper graph of each case). Thus, they were considered as having self-limited acute HCV course. ALT profile was monophasic (ALT declined with no fluctuations) in patients with spontaneous resolution during the study period. Subject #1 was anti-HC positive at baseline and month 6, while at month 12 the result of anti-HCV test was equivocal (near the cut-off value). In patient #2, antibodies to HCV were not detected at tested time-points (baseline, month 6, and month 14) of the study. The latter observations could further indicate clearance of HCV infection in these two individuals.

The remaining six patients who had detectable plasma HCV RNA and abnormal findings of biochemical tests for more than 6 months (range, 8–12 months) developed chronic illness (Fig. 2A–C, upper graph of each case). Antibodies to hepatitis C virus were detectable in this group at baseline as well as the end of the follow-up. Different ALT and HCV RNA patterns were seen among subjects with chronic HCV infection. The following is a description of their biochemical and virological course during the study period.

Patient #3 and patient #4 had acute serum ALT flares (ALT elevation 3 to 9 times previous level) within the first two weeks starting observation (Fig. 2A, upper graph of each case). Subject #3 displayed ALT flare in association with increased viral load by 2-log_{10} that declined immediately after the flare (Fig. 2A, patient #3). The same patient encountered another episode of abrupt elevation of alanine aminotransferase at month 6, although this time it coincided with only minimal changes of HCV RNA ($r_s=0.58$, $P=0.049$). Subject #4 demonstrated relatively stable ALT elevation after acute flare, while HCV RNA load fluctuated from undetectable to $3\text{--}5 \log_{10}$ values nearly the entire follow-up, showing no association with liver enzymes ($r_s=-0.13$, $P>0.05$).

Patients #5 and #6 normalized their ALT levels by

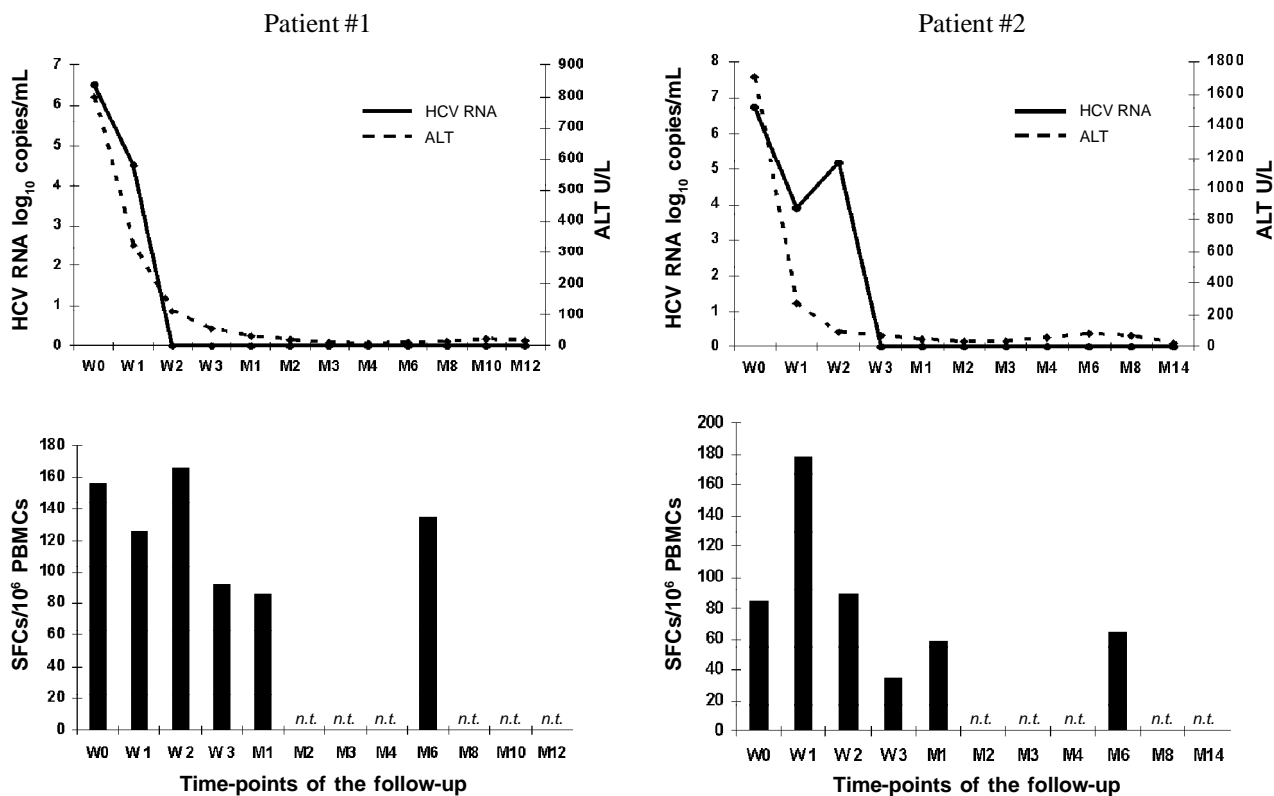


Fig. 1. Dynamics of plasma HCVRNA, serum ALT levels (upper graphs) and virus-specific CD4⁺ T cells that produce IFN- γ (lower graphs) of acute patients (#1 and #2) with self-limited hepatitis C virus infection during the follow-up

Serum alanine aminotransferase (ALT) levels are expressed in U per liter (U/L) (dotted line); plasma HCV RNA values are expressed as HCV RNA log₁₀ copies per mL (solid line). Black bars indicate the frequency of HCV-specific CD4⁺ T cells that produce interferon-gamma (IFN- γ) in response to all four HCV antigens (core, NS3, NS4, NS5). Responses are shown as antigen specific, IFN- γ spot-forming cells (SFCs) per 10⁶ peripheral blood mononuclear cells (PBMCs). W – week; W0 – baseline; M – month; n. t. – not tested.

month 1 and month 2, respectively. Unfortunately, they rapidly relapsed and starting month 3 experienced ALT fluctuations, more pronounced in subject #5 (Fig. 2B, upper graph of each case). HCV RNA was constantly detectable and positively correlated with liver enzymes in the latter subject ($r_s=0.82$, $P=0.001$). The ALT normalization in patient #6 coincided with undetectable HCV RNA at month 2; however, no relationship was found between liver enzymes and viral load over time ($r_s=0.22$, $P>0.05$).

Finally, ALT levels were persistently elevated and displayed a slow decrease pattern in the remaining patients (#7, #8), whereas viral load remained constantly high ($>6\text{-log}_{10}$) and almost stable throughout the study period (Fig. 2C, upper graph of each case). HCV RNA titers did not correlate with ALT levels in these two subjects ($r_s=-0.48$, $P>0.05$ for #7; $r_s=-0.29$, $P>0.05$ for #8).

There was no association between the mean values of plasma viral load and serum ALT in chronically

infected individuals during observation ($r_s=0.4$, $P>0.05$).

HCV-specific CD4⁺ T cell responses

HCV-specific CD4⁺/Th1 cell response was assessed in eight acutely infected subjects using ELISpot assay. We present the strength and kinetic patterns of CD4⁺ T cells mounted to all four viral antigens (core, NS3, NS4, NS5) in resolved and persistently infected patients within the first month and at month 6 during the follow-up (Fig. 1 and Fig. 2A–C, lower graph of each case, Fig. 3).

HCV-specific IFN- γ production was detectable in all acute cases. Patients with self-limited infection demonstrated the strongest virus-specific CD4⁺ T cell responses during initial time-points of the study (at baseline, week 1, and week 2) (Fig. 3). The peak of antiviral T cell numbers in subjects with a favorable course of illness was observed at weeks 1 and 2 of the follow-up. In contrast, chronically infected individuals

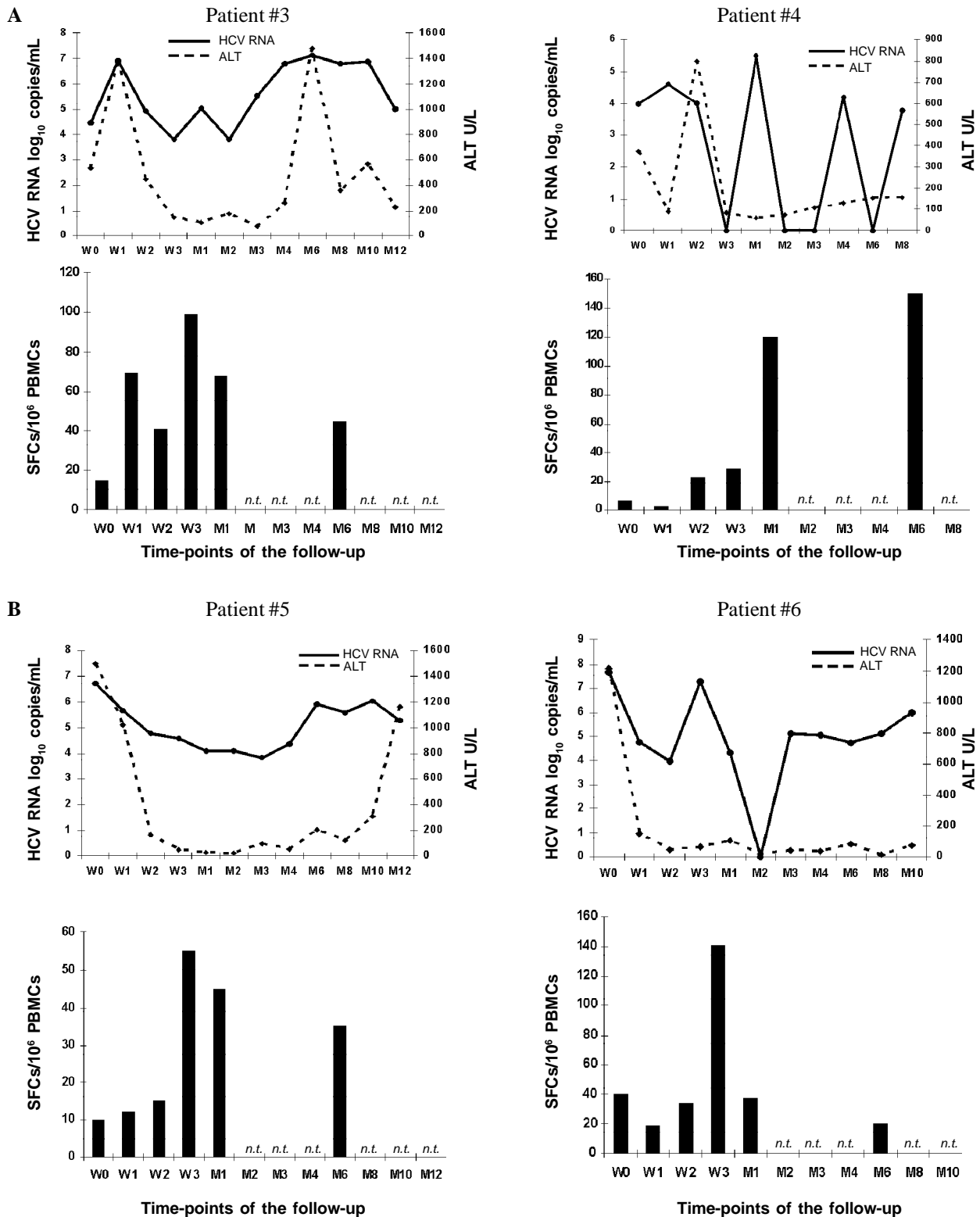


Fig. 2. Dynamics of plasma HCVRNA, serum ALT levels (upper graphs) and virus-specific CD4⁺ T cells that produce IFN- γ (lower graphs) of acute patients (A – patients #3 and #4; B – patients #5 and #6; C – patients #7 and #8) who developed chronic hepatitis C virus infection during the follow-up. Serum alanine aminotransferase (ALT) levels are expressed in U per liter (U/L) (dotted line); plasma HCV RNA values are expressed as HCV RNA log₁₀ copies per mL (solid line). Black bars indicate the frequency of HCV-specific CD4⁺ T cells that produce interferon-gamma (IFN- γ) in response to all four HCV antigens (core, NS3, NS4, NS5). Responses are shown as antigen specific, IFN- γ spot-forming cells (SFCs) per 10⁶ peripheral blood mononuclear cells (PBMCs). W – week; W0 – baseline; M – month; n.t. – not tested.

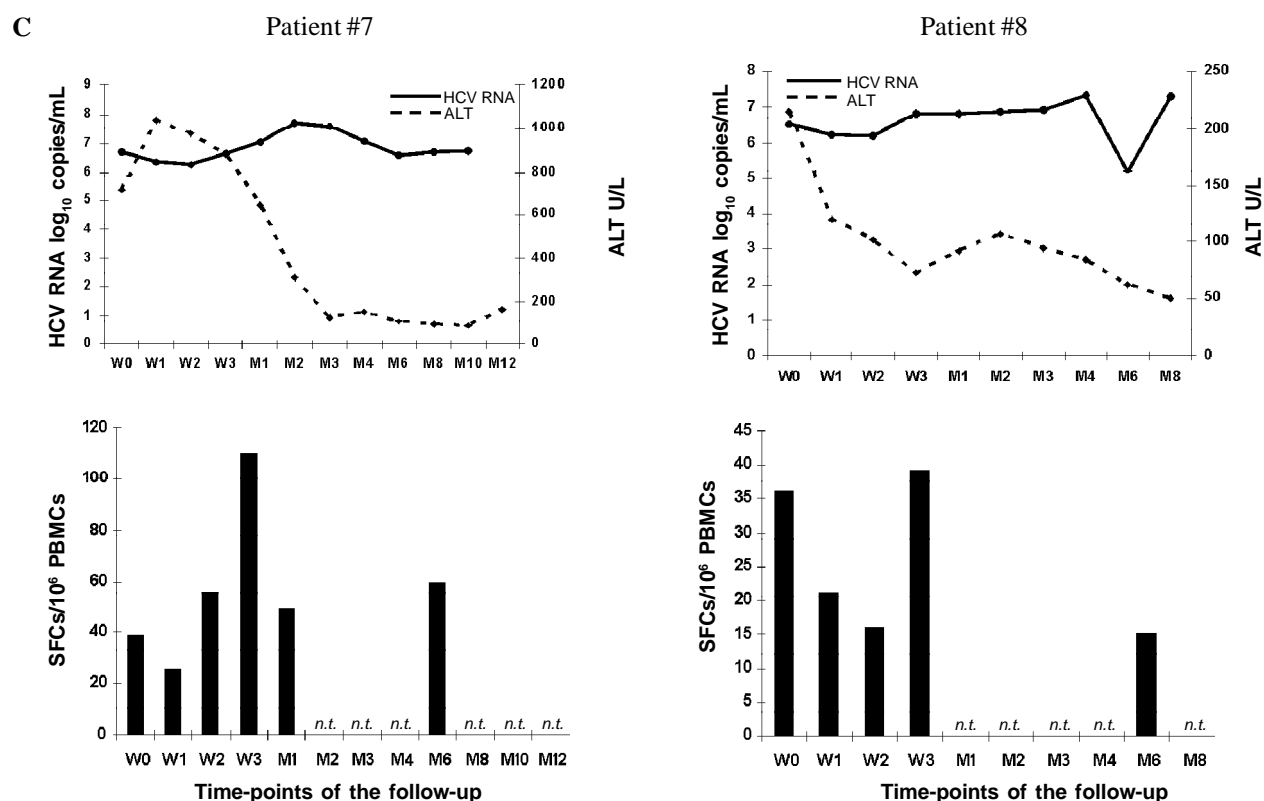


Fig. 2. Continuation (see page 515)

initially displayed a weak HCV-specific CD4⁺ T cell response that increased at weeks 3 and 4 during observation (Fig. 3). At month 6 of the study, CD4⁺ T cell reactivity to hepatitis C virus seemed to remain stronger in resolved cases compared to chronic subjects, although the difference between two groups was not significant at all tested time-points. Within the same period, responses to PHA were uniformly positive in patients with acute hepatitis C (data not shown).

We compared HCV-specific CD4⁺ T cell reactivity and HCV kinetics during primary weeks of infection. Patient #1, for example, lost HCV RNA at week 2 that is at the precisely time point when T cells reached a peak response (Fig. 1, patient #1). Similarly, spontaneously resolved patient #2 demonstrated a decrease of viral load at week 1 that coincided with the strongest T cell reactivity (Fig. 1, patient #2). In contrast, when HCV-RNA increased during the following week 2, virus-specific CD4⁺ T cell response subsided in the same subject. With regard to chronic patients, we observed a negative correlation between their mean T cell numbers and HCV-RNA titers within the first month. Slow decline of viral RNA was associated with rising CD4⁺ T cell numbers in these individuals over the first three weeks, while at week 4, increased plasma HCV-RNA level was accompanied by weaker

cellular immune response ($r_s = -0.9$, $P = 0.037$) (Fig. 4A).

Analysis of resolved patients showed no relationship between HCV-specific CD4⁺ T cell frequency and ALT levels. However, there was a negative correlation between the mean values of HCV-specific T cells and ALT in chronic subjects during initial time points (baseline – week 4) of the follow-up ($r_s = -0.9$, $P = 0.037$) (Fig. 4B).

Discussion and conclusions

Patients with acute hepatitis C represent an ideal model to investigate possible factors responsible for viral clearance and persistence. However, acute phase of HCV infection is rarely identified, because most patients are usually asymptomatic and nearly never seek medical aid. Consequently, the majority of individuals who come to the attention of clinicians are those who are the sickest. Only 15–30% of acute cases result in jaundice that might be preceded and accompanied by slight fatigue, malaise, nausea, and pain in the right upper abdomen (2, 15, 16). Notably, symptomatic patients have been proposed to tend to clear the virus and avoid chronic disease with greater frequency than those who have clinically silent hepatitis C (1, 7, 19). In the present study, eight individuals

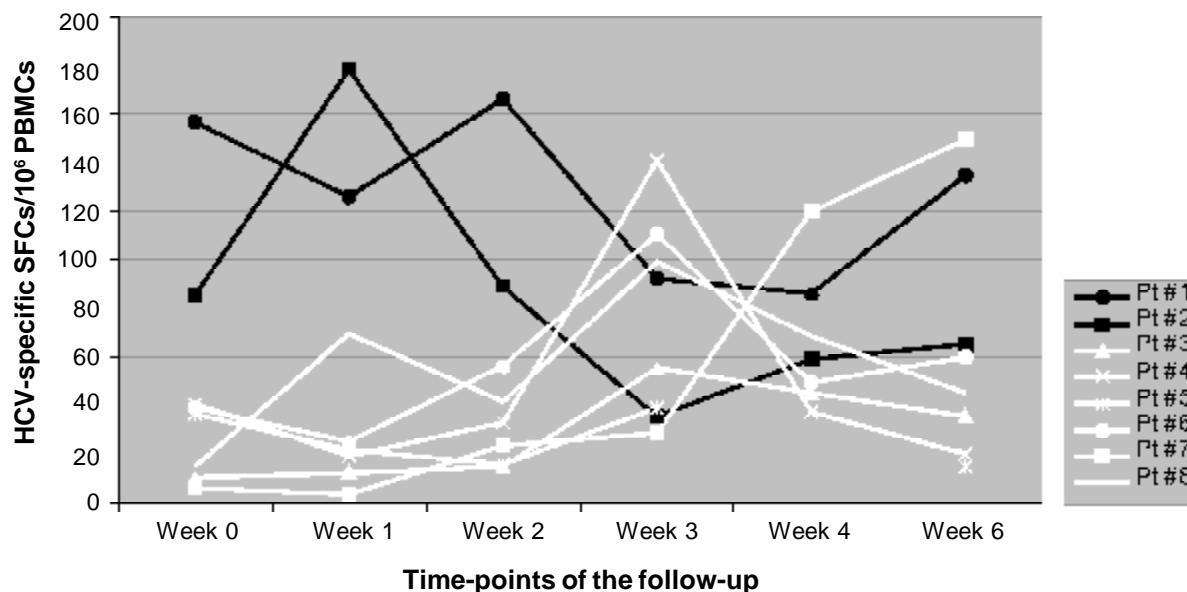


Fig. 3. Kinetics of HCV-specific CD4⁺ T cells that produce interferon-gamma (IFN-g) in patients with self-limited (black line) and chronic (white line) evolution of hepatitis C virus infection during the first month and at month 6 of the follow-up

Numbers of virus-specific CD4⁺ T cells are expressed as antigen specific IFN- γ spot-forming cells (SFCs) per 10⁶ peripheral blood mononuclear cells (PBMCs). Week 0 – baseline; Pt – patient.

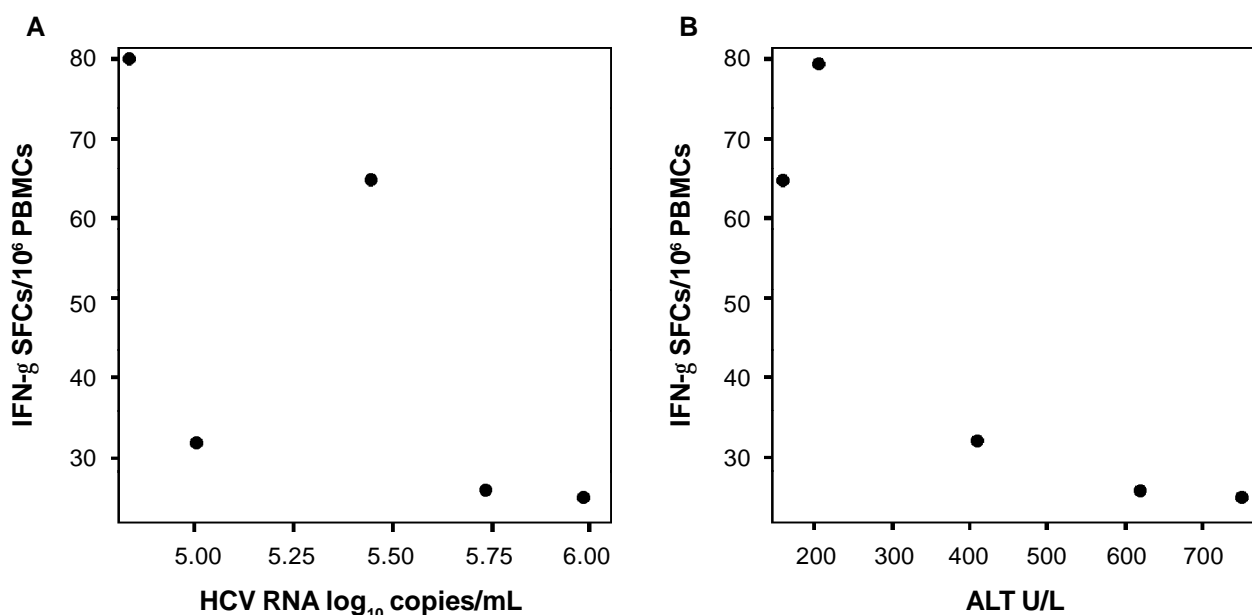


Fig. 4. Relationship between HCV-specific IFN-g producing CD4⁺ T cell frequency and (A) plasma HCV RNA load, (B) serum alanine aminotransferase (ALT) levels in chronically infected patients during initial time-points (baseline – week 4) of the follow-up

A significant negative correlation is found between virus-specific CD4⁺ T (IFN- γ) cell frequency and (A) HCV RNA titers ($r_s = -0.9$, $P = 0.037$) and also (B) ALT values ($r_s = -0.9$, $P = 0.037$).

with acute symptomatic HCV infection were included. Only two of them were able to clear the virus spontaneously, whereas the remaining subjects developed persistent infection. Jaundice and other symptoms are frequently discussed as surrogate markers of vigorous, broad-based antiviral immune response during early

infection (2, 4, 20, 21). According to our findings, HCV-specific CD4⁺ T (IFN- γ) cells were detectable in all acute cases. However, patients with self-limited infection seemed to have stronger antiviral T cell responses than chronic subjects during the first weeks of the follow-up, though the difference was not signi-

ficant between two patient groups, probably because of the insufficient number of individuals analyzed. In addition, baseline levels of serum transaminases (ALT, AST) and bilirubin seemed also to be higher in responders in comparison to subjects with chronic evolution, hence suggesting more severe disease in those leading to the recovery, although again the statistical difference between two groups was not significant, perhaps due to the limited number of participants. In light of these data, it is possible that symptoms and higher initial peak values of biochemical parameters might reflect stronger antiviral immunity related with the disease resolution. Moreover, resolved patients displayed earlier peak responses of CD4⁺ T (IFN- γ) lymphocytes, accompanied by diminished viral titers or undetectable HCV RNA, while persistently infected individuals showed a negative correlation between their mean values of antiviral CD4⁺ T cells and HCV RNA, also ALT levels within the first month of the study, supporting the view that host immune responses might play an important role in the disease process. We demonstrate that early and strong CD4⁺/Th1 cell response may contribute to the viral control and clearance. Of note, in order to achieve long-term viral elimination, vigorous cell-mediated immunity against hepatitis C virus must be permanently maintained in spontaneously recovered individuals (8, 11). Possibly because of a small number of subjects analyzed, we did not find a significant difference of HCV-specific CD4⁺ T (IFN- γ) cell frequency between resolved and chronically infected patients at month 6 of the follow-up, although antiviral T cell numbers seemed to remain higher in subjects with self-limited infection at this time-point. Finally, the breadth of antiviral T cell response was not evaluated in this article; nevertheless, it is necessary to mention that effective resolution of hepatitis C virus typically requires multispecific Th1-type responses directed to the range of HCV antigens (7, 8, 10, 22).

Data obtained in this study support a number of reports indicating that most subjects who eventually recover do so within the first 3 to 4 months of infection (2, 13, 19, 21). According to our results, patients with self-limited illness cleared the virus during the first two weeks from the enrollment to the study or approximately within one month following clinical presentation. Conversely, plasma HCV RNA was constantly detectable or intermittently undetectable among persistently infected individuals until the end of the study. Acute hepatitis C is considered to run a course of approximately 6 months (4, 20, 23). In this respect, repeated evaluation of HCV RNA status during initial 24 weeks after clinical onset might serve as a good

predictor of the disease outcome and could be used as a useful criterion considering the beginning of antiviral treatment. It is therefore clear that a single HCV RNA negative sample during the late phase of acute hepatitis C does not prove resolution of infection. Accordingly, prolonged follow-up with repeated testing for at least 12 months after diagnosis is necessary to prove that the infection has resolved (18, 20). Follow-up is mandatory even after initial spontaneous viral clearance, because recurrent viremia has been described after a period of 4 to 5 months of undetectable HCV RNA in the blood, implying that HCV might persist in the liver or extrahepatic sites after falling below the lower limit of detection in the blood (7, 24, 25).

The ALT profile during acute phase of HCV infection can be highly variable (4, 13, 19) and has been shown to be associated with different outcome of hepatitis C (17). A monophasic ALT profile has been reported to predict recovery, while polyphasic ALT profile is often followed by chronic evolution. Indeed, both resolved patients of the current study rapidly declined ALT levels without any fluctuations, while persistently infected subjects demonstrated constantly elevated liver enzymes with experiencing periods of fluctuating ALT or temporal ALT normalization throughout the study. It should be underlined, however, that serum ALT levels may be extremely diverse in acute hepatitis C and that ALT normalization after acute phase is not a reliable marker of recovery as there are patients who remain viremic despite complete and persistent normalization of ALT (2–5, 14). The mechanisms responsible for the broad clinical spectrum of disease presentations are unclear. In the present study, no relationship between ALT and HCV RNA was found in the majority of acute cases, with the exception of two chronic patients who showed a positive correlation between viral load and liver enzymes during the follow-up. It remains possible that both viral and host immune factors might contribute to the polymorphic features as well as the outcome of HCV infection (26–28).

The rate of chronic outcome is certainly high with HCV in all patient categories, although it can vary depending on a number of cofactors, which include male sex, older age at acquisition of HCV infection, obesity, HIV or HBV co-infection, and alcohol consumption (1, 2, 6). Route of infection, size of inoculum, and host immune status might also influence disease progression (21, 29). Considering a limited number of patients, we did not find a significant difference between possible cofactors of chronicity and the final state of HCV infection, although both patients with self-limited HCV course were females and

seemed to be slightly younger than chronics. In addition, resolved subjects were infected with the genotype 3, which has recently been associated with a favorable outcome of the disease (30). The relationship between female sex and self-limited HCV course has been reported by different authors (3, 14, 20, 23). It is possible that genetic factors or female sex hormones might facilitate HCV clearance in women.

In conclusion, we demonstrate that in most cases acute hepatitis C progresses to chronic disease. Viral clearance within one month after clinical presentation, accompanied by monophasic ALT normalization, could indicate recovery. It seems reasonable to wait at least one month to allow acute symptomatic patients the chance to clear the virus spontaneously, in order to avoid a useless, potentially harmful, and costly treatment of subjects with self-limiting disease. In addition, our findings extend previous observations that the presence of early and strong CD4⁺/Th1 im-

mune response against HCV is likely an important contributing factor for the resolution of HCV infection. Continued immunological analysis of this cohort will determine further kinetics of hepatitis C virus-specific CD4⁺ T cells and the role of Th1 and Th2 cytokine profile during the course of infection. Further studies are needed to understand the underlying mechanisms for the impaired CD4⁺ T cell reactivity in establishment of persistent HCV infection. Finally, supplementary studies should be carried out on a larger group of patients in order to draw definitive conclusions about factors predicting spontaneous viral eradication and parameters responsible for the outcome of acute phase of HCV infection.

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Ūminės virusinės hepatito C infekcijos išnykimas ar progresavimas į lėtinę ligos formą: prospektyviojo tyrimo preliminarūs duomenys

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Raktažodžiai: ūminis hepatitas, hepatito C virusas, imuninis atsakas, T ląstelės, viruso išnykimas.

Santrauka. *Tyrimo tikslas.* Hepatito C virusinė infekcija turi nepaprastai ryškų polinkį progresuoti į lėtinę ligos formą. Deja, hepatito C virusinės infekcijos persistavimo mechanizmai kol kas nepakankamai ištirti. Mes stebėjome ūminės hepatito C virusinės infekcijos natūralią klinikinę, virusologinę ir imunologinę eigą, kad galėtume identifikuoti galimus veiksnius, prognozuojančius ligos spontaninį išnykimą, taip pat įvertinti ankstyviausius veiksnius, galinčius turėti įtakos infekcijos savaiminiam išnykimui ar chronizavimuisi.

Medžiaga ir metodai. Ištyrėme aštuonis ligonius, kuriems buvo diagnozuotas simptominis ūminis hepatitas C. Pacientus stebėjome 8–14 mėnesių. Tyrimo metu pacientai nebuvo gydomi antivirusiniais vaistais. Stebėjimo metu įvertinome biocheminius, virusologinius ir imunologinius tyrimo dalyvių duomenis. Hepatito C virusinės infekcijos RNR kiekis kraujo plazmoje nustatytas TaqMan® tikrojo laiko polimerazės grandininės reakcijos (PGR) metodu. Virusui specifinių CD4⁺ T ląstelių, sekretuojančių gamainterferoną (IFN-γ), skaičius nustatytas „ELISpot“ metodu.

Rezultatai. Du tyrime dalyvavę pacientai pasveiko spontaniškai, o likusiems šešiams ligoniams pasireiškė lėtinis hepatitas C. Pasveikusiems tiriamiesiems virusas savaime išnyko per pirmąsias 14 stebėjimo dienų, po kurių ALT fermento aktyvumas greitai sunormalėjo. Lėtinė infekcija pasižymėjo persistuojančia viremija ar laikinai neigiama PGR reakcija bei polifazine ALT aktyvumo eiga per visą stebėjimo laikotarpį. Spontaniškai pasveikusių tyrimo dalyvių grupėje stipriausias virusui specifinių CD4⁺ T (IFN-γ) ląstelių imuninis atsakas pastebėtas per pirmąsias stebėjimo savaites. Tuo tarpu ligoniams, kuriems pasireiškė lėtinė hepatito C forma, tyrimo pradžioje nustatytas mažiausias virusui specifinių CD4⁺ T (IFN-γ) ląstelių skaičius.

Išvados. Ūminė hepatito C virusinė infekcija yra linkusi progresuoti į lėtinę ligos formą. Ūminės hepatito C infekcijos RNR išnykimas per pirmąją ligos mėnesį nuo simptomų pasireiškimo, lydimas monofazinio ALT aktyvumo sunormalėjimo, galėtų prognozuoti savaiminį pasveikimą. Tikėtina, kad ankstyvas ir stiprus virusui specifinis CD4⁺/Th1 imuninis atsakas turi įtakos spontaniniam viruso išnykimui.

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