Viral Dynamics in Hepatitis C*

David Kershenobich MD PhD and Christian Heinrich Henonin MD

Department of Gastroenterology, National Institute of Medical Sciences and Nutrition Salvador Zubirán, Mexico City, Mexico

Key words: hepatitis C, viral dynamics, chronic liver diseases, chronic hepatitis C, hepatitis C natural history

Abstract

The hepatitis C virus is an enveloped positive-sense single-stranded RNA virus, which has been classified into 6 major genotypes and over 100 subtypes. HCV replicates mainly in the hepatocyte. Recently, infectious HCV cDNA clones have been generated. Despite evidence that innate and adaptative humoral and cellular immune responses are activated as part of an antiviral defense, HCV has a remarkable ability to establish persistent infection. The analysis of viral kinetics using mathematical modeling shows a relative steady state without treatment, while an immediate biphasic HCV decline occurs in blood during successful treatment, the latter being predictive of clearance of HCV by the end of treatment.

IMAJ 2001;3:360-363

The virus

The hepatitis C virus is an enveloped positive-sense single-stranded RNA virus of approximately 9,600 nucleotides that represent a separate genus in the Flaviviriade family [1,2]. The viral genome encodes a single polyprotein that is co- and post-transitionally processed by cellular and viral proteases to produce specific viral gene products. It has a single open reading frame about 9,000 nucleotides in length. The ORF is flanked at each terminus by untranslated regions. Translation of the HCV ORF produces a polyprotein of about 3,000 amino acids that is cleaved into at least 10 structural and non-structural proteins by a combination of host and viral proteases. A distinctive characteristic of HCV is its genetic heterogeneity [3,4]. Thus, HCV isolates from around the world have been classified into 6 major genotypes and over 100 subtypes. The genomes of the most different HCV isolates differ by up to 35%. There are clear differences in the genotype distribution. Genotypes 1a, 1b, 2a, 2b, 2c and 3a account for about 90% of the HCV infections in North and South America, Europe, Russia, China, Japan and Australia/New Zealand [5,6]. In Mexico genotype 1b accounts for 68% of the HCV isolates, subtype 1a for 9% and subtype 2a and 2b, for 9% of HCV infections [7].

HCV replicates in hepatocytes of infected patients. It is possible that HCV can replicate at low levels in peripheral blood mononuclear cells and bone marrow cells of infected patients. Recent advances in the field of HCV research – the generation of infectious HCV cDNA clones and of an HCV replicon – will permit more detailed studies of the molecular biology of HCV and could facilitate the establishment of *in vitro* replication systems. Infectious cDNA clones have now been generated for HCV strains of genotypes 1a, 1b and 2a. Genomic RNA transcripts synthesized *in vitro* from full-length HCV cDNA clones were infectious *in vivo* when inoculated into the liver of chimpanzees. The HCV infection generated by transfection of chimpanzees did not differ significantly from the infection observed in animals infected intravenously with the original virus [8,9].

The host response

Despite evidence that innate and adaptative, humoral and cellular immune responses are activated as part of the antiviral defense and that the adaptative immune response is targeted against immunogenic sequences in all viral proteins, HVC has a remarkable ability to establish persistent infections characterized by an increase in genetic diversity in the form of viral quasispecies. Thus, the virus appears to have evolved strategies to not induce, overcome or evade efficient immune responses of the host [10].

HCV-specific antibodies are generally detectable 7 to 31 weeks after infection and are targeted against epitopes in all viral proteins. The existence of neutralizing antibodies to HCV and their role in the outcome of infection is controversial. Long-lasting protective antibody responses have not been demonstrated [11].

An early vigorous and multispecific immune response of CD4+ and CD8+ T cells has been associated with self-limited HCV infection and viral clearance. In the blood of chronically

* Presented at the Mexico-Israel Symposium "Clinic and Science: Interfaces," 15–16 February 2001, Mexico City

HCV = hepatitis C virus
ORF = open reading frame

infected patients, the frequency of HVC-specific cytotoxic T lymphocytes is rather low. For example, T cells specific for two HCV NS3 epitopes constitute between 0.01 and 2% of all peripheral blood CD8+ T cells in chronically infected patients [12]. At the site of inflammation in the infected liver the frequency of CD8+ T cells was found to be at least 30-fold higher than in the blood [12]. All of the intrahepatic HCV NS3-specific CD8+ T cells were activated, and the predominant phenotype of all antigen-specific and non-specific liver infiltrating cells has been described as Th1/Tc1 type producing interferon-gamma [13]. While activated peripheral blood CD8+ T cells are continuously trapped in the liver regardless of their antigen specificity, the majority of liver-infiltrating lymphocytes is HCV non-specific [13], and intrahepatic compartmentalization of distinct T cell subsets as well as distinct HCV quasispecies have been observed. Once activated CD8+ T cells are trapped in the liver they undergo activation-induced cell death, presumably due to T cell activation in the absence of sufficient co-stimulation. Under such conditions apoptosis occurs within 18 hours [14]. Thus, activated cells are continuously recruited from the peripheral blood, and the loss of lymphocytes by intrahepatic sequestration and apoptosis has been estimated to be as high as 2×10^8 cells, i.e., 0.1% of the total body lymphocytes per day [15].

Immune-mediated liver disease is thought to be initiated by these HCV-specific liver-infiltrating T cells, but are amplified by antigen non-specific cells. Long-standing immune-mediated liver injury may eventually result in liver cirrhosis and hepatocellular carcinoma. Presentation of HCV antigens on infected hepatocytes, recognition by CTLs and induction of liver injury seems to be enhanced by HCV-induced expression of HLA A, B, C and ICAM-1 molecules.

Some of the mechanisms that allow HCV to persist despite the humoral and cellular responses include the possibility that HCV up-regulates major histocompatibility complex expression on infected cells, and that it has found additional mechanisms to avoid infection of specific T cell responses, low frequency of HCV-specific T cells, HCV sequence variations and mutations in T cell epitopes and flanking regions, lack of susceptibility of HCV to T cell cytokines, and HCV interference with antigen processing [16,17].

The disease

Accurate determination of the natural history of hepatitis C is hampered by the fact that the initial onset of infection is usually devoid of signs and symptoms, that progression from acute to chronic hepatitis is extremely common, and that the duration to development of end-stage liver disease may exceed 30 to 40 years. It is widely accepted that about 15% of acutely infected persons recover spontaneously from HCV infection. There are emerging data, however, to suggest that 25% to 45% may recover spontaneously. These data are derived largely from studies in young people, particularly young women [18,19].

Direct determination of the incidence of new infection is unfeasible, since most acute HCV infections are asymptomatic and clinical disease is under-reported. Moreover, the assumption that infection always results in seroconversion may not be true for a minority of cases. HVC-RNA has been found in up to 10% of patients with chronic hepatitis who are persistently anti-HCV negative [20]. The mechanisms of chronicity are not as yet understood. The course of chronic hepatitis C with alanine aminotransferase elevations is often unpredictable in the individual patient. In a number of patients it progresses slowly to more severe and active liver disease, with increasing fibrosis and ultimately with transition to cirrhosis. Cirrhosis may become complicated by severe sequelae, including hepatocellular carcinoma. Progression may take years or decades. In other patients, the liver disease remains stable in a mild or intermediate state without significantly worsening. The speed of the advance to end-stage liver disease remains controversial. When the degree of hepatic fibrosis was related to the known duration of HCV infection, chronic HCV carriers could be classified as rapid, intermediate, and slow "fibrosers" – with an estimated time interval between infection and development of cirrhosis of 3–10 years, 15–30 years, and more than 50 years, respectively; the number of patients in each group was similar [21]. It should be noted that the rate of fibrosis progression is also affected by other factors such as male gender, alcohol consumption, and acquisition of HCV after the age of 45 years. Several questions have yet to be addressed: Is advancing disease inevitable? Does progression advance linearly? Is progression immunologically driven? What co-factors contribute to progression?

Chronic HCV infection has been associated with a variety of extrahepatic manifestations. We recently explored the contribution of class II genes to the expression of autoimmunity in chronic HCV patients and confirmed that DQB locus is involved in the genetic control of autoantibody production. Moreover, DRB1 0301 is partly responsible for the chronic process unchain by HCV [unpublished observation].

Viral kinetics

The analysis of viral kinetics as a tool for the clinician has only recently become part of the armamentarium for the study and treatment of patients with chronic hepatitis C. The knowledge that the half-life of HCV virus is about 2.7 days and that HCV production on a daily basis can be as high as 1 x 1,200 virions (one trillion) per day [22] poses some intriguing questions. What is the effect of fluctuations in viral load during the natural history in hepatitis C? What is the impact of the spread of the virus to uninfected cells? What is the impact of the different effective drugs against viruses? How do they impact on the steady state of viral loads? Does clinical heterogeneity in disease progression reflect either viral heterogeneity or variations in host response?

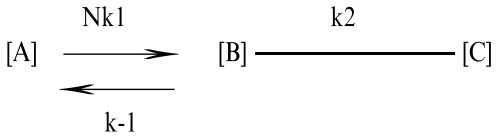
CTL = cytotoxic T lymphocytes

The fluctuations in viral load during the natural history of hepatitis C are poorly understood. The half-life of HCV genotype 1 is 2.7 hours, while that for genotype 2 is 1.8 hours. The half-life differences between genotypes 1 and 2 may be part of the reason why genotype 1 is more refractory to current treatments than other genotypes.

The complex genetic composition of quasispecies means that there are subtle genetic differences among different virus particles within the same person. Each type of viral infection has complex dynamics that become more apparent in response to treatment. This refers to mutations that may be selected for under the pressure of antiviral drugs. Viruses with such mutations will eventually become the dominant species, even in the presence of antiviral drugs.

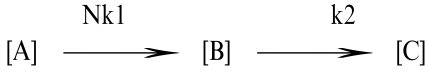
The rate of dynamic viral change may vary in different body compartments. Evidence for HCV replication outside the liver has been found in the lymph nodes, pancreas, adrenal glands, thyroid, bone marrow and spleen [23], although the amount of HCV produced at these sites appears to be relatively low. The clinical significance of extrahepatic HCV is not fully understood. As hepatitis C may occur associated with other viruses such as human immunodeficiency virus, interaction between different viruses reflects directly on the viral HCV load. For example, average HCV RNA levels (viral load) in people co-infected with HIV has been reported to be as high as 16.8 million copies/ml. Factors associated with lower than average HCV RNA levels in HIV-negative patients include younger age at infection and female gender.

An analytical model of the infectious cycle of HCV *in vivo* has been described by Zeuzem et al. [24], in which compartment A represents the pool of HCV-infected and non-infected hepatocytes as well as extrahepatic replication sites, and B(rNA) denotes the HCV RNA concentration in serum and is a fictitious degradation compartment.



HCV is produced in infected hepatocytes and subsequently released into the systemic circulation at rate constant Nk1, where N is the number of virions produced per infected cell, and In2/k1 is the half-life of virus-producing cells as well as that of virus release. Degradation of free virus from the blood occurs at a rate constant k2. Because antibody-complex virions may have a rate of degradation different from that of non-complex virions, k2 must be interpreted as a combination of antigen-specific and non-specific processes. During steady-state conditions before antiviral treatment, adjacent and distant hepatocytes become infected at a rate constant k-1. However, virus uptake by previously uninfected (*de novo* infection) or infected (superinfection) hepatocytes cannot be discriminated and transformation of non-infected into virus-producing hepatocytes cannot be measured.

HCV RNA decline after initiation of therapy is obtained, assuming that the predominant antiviral effect is the inhibition of *de novo* infection of susceptible cells (k1 and k2 greater than 0., k-1 = 0). The rate of HCV elimination from serum following initiation of antiviral therapy is then determined by two processes: namely, the clearance of HCV RNA per se and the elimination or suppression of virus-producing cells. Thus, viral RNA data are fitted to a three-compartment sequential reaction model according to:



where k1 and k2 denote the rate constants associated with increase and decrease of viral RNA concentration B, respectively. Accordingly, the differential change in the virus concentration B shall **obey** the hypothesis:

$$\frac{db}{dt} = Nk1A \quad k2B$$

Integration requires that an assumption on the time dependence of A be made, as well as an initial condition for the compartmental population be given. Let

$$A(t) = A(0)\exp(-k1t)$$

indicating the concentration of virus-producing cells leveling off at rate dA/dt = -k1A after the initiation of treatment.

Using this model it has been shown that higher interferon doses accelerated viral clearance from serum, as in patients treated with 3 x 6 **MU** IFN[1] ($t_{1/2} = 0.23 \pm 0.15$) compared with patients treated with 3 x 3 **MU** IFN per week (0.67 ± 0.36 days). Ribavarin 14 mg/kg/day, however, has no synergistic antiviral effect in the treatment of chronic hepatitis C with 3 x 6 MU IFN per week [25].

Although several virus and host-related predictive factors for the response to IFNα have been defined in patients with chronic hepatitis C, no pretreatment parameter can definitively predict the response to antiviral treatment. Assessment of the initial response by quantification of serum HCV RNA before and 4 weeks after initiation of therapy may be a clinically applicable and reliable parameter to predict long-term response. Recent results suggest that IFN treatment can be terminated after 4 weeks in patients, with a decrease in HCV RNA levels of less than 3 log, when apparent HCV eradication is considered the therapeutic target. The predictive value of the delta HCV RNA

HIV = human immunodeficiency virus

IFN = interferon

clearly exceeds the significance of HCV genotype and pretreatment viremia as predictors of successful IFN treatment [26].

A special area of interest with relation to viral dynamics of hepatitis C is liver transplantation. These patients are ideally suited to provide insights into the mechanisms of HCV pathogenesis, since hepatic HCV replication is terminated at the time of the removal of the infected liver, and the clinical setting following reperfusion of the engrafted liver provides a model for monitoring dynamic processes of graft reinfection. Accordingly, in a study involving nine patients with HCV-positive cirrhosis following orthotopic liver transplantation, there was a rapid decline in HCV RNA levels from 3.1 $\pm$ 1.3 x 100,000 copies/ml preoperatively, which decreased to 0.15 $\pm$ 0.6 x 100,000 copies/ml on the first postoperative day and 0.16 $\pm$ 0.6 x 100,000 copies/ml on the second (mean viral half-life 4.0 $\pm$ 0.5 h). Thereafter, HCV RNA levels increased in all but one patient, and by postoperative day 8 reached 3.6 $\pm$ 1.3 x 100,000 copies/ml, exceeding the preoperative levels irrespective of whether or not immunosuppressive therapy was used. These findings indicate that the half-life of HCV is quite short, and that extrahepatic viral replication contributes little to the total virus pool in serum. Furthermore, the marked HCV replication beginning as early as the third postoperative day strongly suggests that the liver graft is rapidly reinfected by HCV after transplantation [27].

References

1. Purcell RH. Hepatitis C virus (Flaviviriade). In: Encylopaedia of Virology. London: Academic Press Ltd., 1999:657–63.
2. Reed KE, Rice CM. Molecular characterization of hepatitis C virus. In: Reesink HW, ed. *Curr Stud Hematol Blood Transf*, No 62, Basel: Karger,1998:1–37.
3. Bukh J, Emerson SU, Purcell RH. Genetic heterogeneity of hepatitis C virus and related viruses. In: Rizzeto M, Purcell RH, Gerin JL, Verme G, eds. Viral Hepatitis and Liver Disease. Turin: Minerva Medica, 1997:167–75.
4. Clarke B. Molecular virology of hapatitis C virus. *J Gen Vir*ol 1997;78:2397–410.
5. Maertens G, Stuyevr L. Genotypes and genetic variation of hepatitis C virus. In: Harrison TJ, Zuckerman A, eds. The Molecular Medicine of Viral Hepatitis. Medical Science Series. Chichester, UK: John Wiley & Sons Ltd, 1997:183–233.
6. World Health Organization. Hepatitis C: global prevalence. *Wkly Epidemiol Re*c 1997;72:65–72.
7. Lisker Melman L, Kershenobich D, Borras Cuesta F, Riezu-Boj JI, Rodriguez-Lopez M, Sixtos S, Solana S, Salazar L, Bonder D, Zapata L. Distribucion genotipica del virus de la hepatitis C en un hospital de tercer nivel de la Ciudad de Mexico. *Rev Gastroenterol Mex* 1995;60:S–70
8. Major ME, Mihalik K, Fernandez J, Seidman J, Kleiner D, Kolykhalov AA, Rice ChM, Feinstone SM. Long term follow-up of chimpanzees inoculated with the first infectious clone for hepatitis C virus. *J Virol* 1999;73:3317–25.
9. Yangai M, Purcell RH, Emerson SU, Bukh J. Hepatitis C virus: an infectious molecular clone of a second major genotype (2a) and lack of viability of intertypic 1a and 2a chimeras. *Virology* 1999;262:250–63.
10. Farci P, Shimda P, Coiana A, Diaz G, Peddies G, Melpolder JC, Strazzera A, Chien DY, Munoz SJ, Balestrieri A, Purcell RH, Alter HJ. The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. *Science* 2000 288:339–44.
11. Houghton M. Strategies and prospects for vaccination against the hepatitis C viruses. *Curr Top Microbiol Immunol* 2000;242:327–40.
12. He XS, Rehermann B, Lopez-Labrador FX, Biosvert J, Cheung J, Mumm J, Wedemeyer H, Berenguer M, Wright TL, Davis MM, Greenberg H. Quantitative analysis of hepatitis C virus-specific CD8(+) T cells in peripheral blood and liver using peptide-MHC tetramers. *Proc Natl Acad Sci USA* 1999;96:5692–7.
13. Bertoletti A, D'Elios MM, Boni C, De Carli M, Zingego AL, Durazzo M, Missale G, Penna A, Fiaccadori F, Del Prete G, Ferrari C. Different cytokine profiles of intrahepatic T cells in chronic hepatitis B and hepatitis C virus infections. *Gastroenterology* 1997;112:193–9.
14. Mehal WZ, Juedes AE, Crispe IN. Selective retention of active CD8+ t cells by the normal liver. *J Immunol* 1999;163:3202–10.
15. Nuti S, Rosa D, Valiante Nm, Saletti G, Caratozzolo M, Dellabona P, Barnaba V, Abrignani S. Dynamics of intra-hepatic lymphocytes in chronic hepatitis C: enrichment for Valpha24+ T cells and rapid elimination of effector cells by apoptosis. *Eur J Immunol* 1998;28:3448–55.
16. Chang KM, Rehermann B, McHutchison JG, Pasquinelli C, Southwood S, Sette A, Chisari FV. Immunological significance of cytotoxic T lymphocyte epitope variants in patients chronically infected by the hepatitis C virus. *J Clin Invest* 1997;100:2376–85.
17. Weiner A, Erickson AL, Kansopon J, Crawford K, Mutchmore E, Hughes A, Houghton M, Walker CM. Persistent hepatitis C virus infection in a chimpanzee is associated with emergence of a cytotoxic T lymphocyte escape variant. *Proc Natl Acad Sci USA* 1995;92:2755–9.
18. Alter MJ, Kruszon-Moran D, Nainan OV, Mc Quillan GM, Gao F, Mayer LA, Kaslow RA, Margolis HS. The prevalence of hepatitis C virus infection in the United States, 1998 through 1994. *N Engl J Med* 1999;341:556–62.
19. Seeff LB. The natural history of hepatitis C – a quandary [Editorial]. *Hepatology* 1998;28:1710–12.
20. McHutchison JG, Kuo G, Houghton M, Choo QL, Redeker AG. Hepatitis C virus antibodies in acute icteric and chronic non-A, non-B hepatitis. *Gastroenterology* 1991;101:1117–19.
21. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997;349:825–32.
22. Neumman AU, Lam NP, Dahari H, Gretch DR, Wilwy TE, Layden TJ, Perelson AS. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 1998;282:103–7.
23. Laskus T, Radkowski M, Wang LF, Vargas H, Rakela J. Search for hepatitis C extrahepatic replication sites in patients with acquired immunodeficiency syndrome: specific detection of negative strand viral RNA in various tissues. *Hepatology* 1998;28:1398–401.
24. Zeuzem S, Schimdt JM, Lee J-H, Rüster B, Roth WK. Effect of interferon alfa on the dynamics of hepatitis C virus turnover in vivo. *Hepatology* 1996;23:366–71.
25. Zeuzem S, Schmidt JM, Lee J-H, Von Wagner M, Teuber G, Roth WK. Hepatitis C virus dynamics in vivo: effect of ribavirin and interferon alfa on viral turnover. *Hepatology* 1998;28:245–52.
26. Zeuzem S, Lee J-H, Franke A, Rüster B, Prümmer O, Herrmann G, Roth WK. Quantification of the initial decline of serum hepatitis C virus RNA and response to interferon alfa. *Hepatology* 1998;27:1149–56.
27. Fukumoto T, Berk T, Ku Y, Bechstein WO, Knoop M, Lemmens H-P, Lobeck H, Hopf U, Neuhaus P. Viral dynamics of hepatitis C early after orthotopic liver transplantation: evidence for rapid turnover of serum virions. *Hepatology* 1996;24:1351–4.

Correspondence: Dr. D. Kershenobich, Departamento de Gastroenterología, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Tlalpan C.P. 14000, México D.F-México. Phone: (52-5) 573-3418, Fax: (52-5) 655-0942,
email: kesd@quetzal.innsz.mx