Perspective

The Fragile Relationship Between Hepatitis C Virus and Its Human Host

Based on viral dynamics and replicative fidelity alone, suppression of hepatitis C virus (HCV) should be a substantially greater challenge than suppression of HIV. Factors underlying the greater than expected responsiveness of HCV to direct-acting antiviral (DAA) drugs include the vulnerability of HCV during acute infection, acceleration of second-phase viral decay kinetics with increased anti-HCV regimen potency, and the effect of DAA treatment in upsetting the equilibrium between the virus and the host immune system. Several potential mechanisms might explain the considerable vulnerability of HCV to potent antiviral therapy. It is possible that anti-HCV treatment destabilizes HCV replication complexes, thereby permitting cure of infected cells, and that with the rapid reduction of HCV within the hepatocyte, mechanisms by which HCV evades the innate and adaptive immune responses are undermined, thus enhancing the antiviral effect of potent anti-HCV regimens. This article summarizes a presentation by Robert T. Schooley, MD, at the IAS–USA continuing education program held in New York, New York, in June 2013.

Keywords: *HCV, hepatitis C, innate, adaptive, immunity, immune evasion, direct-acting antivirals, treatment, replication complex, host interaction*

The response of hepatitis C virus (HCV) to direct-acting antivirals (DAAs) has been better than might be expected based on experience with antiretroviral chemotherapy, reflecting aspects of HCV biology and the relationship between HCV and the host that are now being more fully appreciated. To understand some of the initial pessimism regarding the length of time necessary to develop interferon alfa–free regimens following the availability of new, small-molecule anti-HCV drugs, it is useful to review what has been learned from the study of HIV biology.

Lessons From HIV Biology

In the early days of studying the viral dynamics of HIV, made possible by the introduction of potent antiretroviral drugs, it was observed that the level of virus in plasma could be reduced by more than 100-fold within several days of exposure to a potent drug. This was a surprising finding as HIV replication was initially believed to be slow paced. With these findings it became clear that HIV is a virus with rapid turnover that destroys a large number of lymphocytes each day and that the beneficial impact of antiretroviral therapy could be directly traced to the reduction of T cell turnover associated with suppression of viral replication. From these observations, virion production was estimated at 4×10^8 to 3×10^{10} per day.

A virus that replicated this rapidly, particularly an RNA virus with an error-prone polymerase, would be expected to readily generate resistant mutants. HIV mutants differing from wild type by 5 point mutations were expected to occur as the replication level approached the estimated daily range of virion production. Data such as these contributed to the prediction that control of viral replication would require 3 potent antiretroviral drugs to overcome 5 to 8 collective resistance mutations—a prediction that was borne out in the development of potent antiretroviral regimens.

It was first observed in the Merck 035 study[1] that a 3-drug combination of zidovudine, lamivudine, and indinavir provided sufficient potency and mutational barriers to suppress HIV below detection limits for a prolonged period. Treatment with the 2-drug combination of zidovudine and lamivudine had less potency and a lower barrier to resistance than the 3-drug combination and was associated with virologic breakthrough within several weeks. Initiating therapy with indinavir and then adding zidovudine and lamivudine resulted in a failure to suppress virus to undetectable levels, because the initial exposure to the single drug indinavir resulted in selection of resistant mutants.

Studies of HCV kinetics have shown that 100- to 3000-fold (4×10^{10} to 1×10^{13}) more HCV virions are produced per day than is seen with HIV infection.[2] The HCV polymerase is also error-prone, but its function differs from that of the HIV polymerase in that it must copy a positive strand to a negative strand and then a negative strand to a positive strand each time a new viral particle is produced, thereby giving the enzyme twice the opportunity to introduce mutations with each replicative cycle than in the case of the reverse transcriptase of HIV-1. The high replication rate of HCV results in a tremendous genetic heterogeneity. On a global scale, HCV is more than 10 times more genetically diverse than HIV; HCV genotype 1 alone is as diverse as all clades of HIV. Further contributing to genetic diversity is the fact that HCV has no overlapping reading frames. HIV, on the other hand, has overlapping reading frames, placing substantially greater evolutionary constraints on genetic diversification. The implication of these considerations is that based solely on calculations of viral dynamics and replicative fidelity, HCV suppression should pose a substantially greater challenge than was experienced with HIV.

Dr Schooley is Professor and Vice Chair of the Department of Medicine and Head of the Division of Infectious Diseases at the University of California San Diego in La Jolla, California.

Responsiveness of HCV

Why might HCV be more responsive to DAA therapy than expected based on viral dynamics and fidelity modeling? Factors contributing to this greater responsiveness include the vulnerability of HCV during acute infection, the acceleration of second-phase viral decay kinetics with increased anti-HCV regimen potency, and the effect of DAA treatment in upsetting the equilibrium between HCV and innate and adaptive immunity.

Vulnerability During Acute Infection

One factor contributing to the responsiveness of HCV to potent therapy is that HCV appears to be precariously balanced in the host. HCV infection is cleared during acute infection in approximately 15% to 40% of individuals. Much of the host's ability to clear HCV infection is determined by polymorphisms in the IL28B gene, which is active in regulating the endogenous interferon axis[3] and other aspects of innate immune response. During acute infection, HCV is highly responsive and sensitive to interferons and other elements of innate immune response.

A study of cytokine expression in 18 patients who cleared acute infection and in 35 patients who developed chronic infection revealed that viral clearance is characterized by high levels of tumor necrosis factor alpha, a proinflammatory cytokine that is an important primer of the adaptive immune system and cellular immune response.[4] Persistent HCV infection was characterized by increased levels of interleukin (IL)-2, which is active in stimulating T regulatory cells that downregulate the cellular immune response. Thus, at the time of first viremia, the host is already initiating either a scenario to clear the infection or a program of immune modulation that will allow the virus to persist. Persistence was also characterized by increased levels of IL-10 and IL-13, which skew immune response toward T helper 2 (T_H2) cells, resulting in decreased T cell activation. Treatment with interferon alfa during the acute phase of HCV infection markedly augments viral clearance, which further emphasizes the interferon responsiveness of HCV.

Such observations indicate that the natural history of HCV infection, unlike that of HIV infection, includes a fragile early phase during which the virus is attempting to establish itself in the liver. Once established, the virus stays within the infected liver cells for the remainder of the host's life. Unlike HIV, HCV cannot retreat to latently infected cells with integrated, unexpressed viral DNA. As noted above, HCV must replicate continuously within infected liver cells, displaying its proteins and RNA within these cells in the presence of potent innate and adaptive immune responses.

Acceleration of Second-Phase Kinetics With Increased Regimen Potency

Initial studies of HCV kinetics during treatment with interferon alfa revealed a biphasic decay of the virus from the plasma. The first, steeper phase was thought to correspond to cessation of virus production and release from infected cells, and the second, more gradual phase was thought to correspond to the turnover of infected liver cells.[5] Sustained virologic response (SVR) would be expected with the death of the last infected hepatocyte. In this model, increased effectiveness at turning off virus production would produce steeper first-phase decay but would not be expected to affect the dynamics of second-phase decay. However, studies assessing HCV decay using interferon alfa–based regimens combined with the DAA telaprevir indicated that the increased reduction in viral replication with the addition of telaprevir produced not only steeper first-phase viral RNA decay but also steeper second-phase decay (Figure 1).[6] This suggests a linkage between second-phase decay and how rapidly virus production is turned off.

The understanding of HCV kinetics has been improved by examining the response of gamma-interferon–induced protein (IP) 10 (IP-10) to HCV infection. IP-10 is a chemokine that attracts monocytes, T cells, and natural killer cells. Produced by a number of cells, including hepatocytes, IP-10 is upregulated by HCV infection and by the administration of interferon alfa. When HCV infects hepatocytes in tissue culture or in a human host, there is a rapid increase in levels of IP-10 RNA and IP-10 protein, which remain elevated during chronic infection and return to baseline with viral clearance following effective therapy. Higher pretreatment levels of IP-10 predict a lower likelihood of efficacy with an interferon alfa–based regimen. A greater fold change in plasma IP-10 levels from baseline immediately after the initiation of interferon alfa–based therapy predicts a better treatment response.

Recent studies of IP-10 report a correlation between low levels of IP-10 at

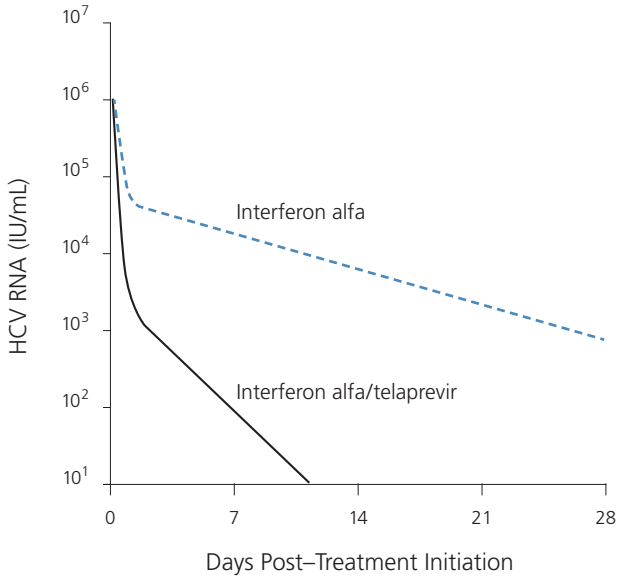

Figure 1. Difference between first- and second-phase viral decay of hepatitis C virus (HCV) during treatment with interferon alfa alone and with the addition of the direct-acting antiviral drug telaprevir. Adapted from Guedj and Perelson.[6]

baseline and IL28B CC genotype, both of which are favorable for HCV clearance.[7-10] The IP-10 response to anti-HCV treatment may reflect the clearance of HCV replication complexes from infected cells, thus explaining the increase in slope of second-phase decay with the use of more potent HCV treatment. These studies show that initiation of treatment with DAAs is associated with a rapid decline in IP-10 levels.[11] The decline was biphasic both in patients whose previous interferon alfa–based therapy had failed and in treatment-naive patients. Between weeks 1 and 2 of treatment, the change in IP-10 level was more pronounced in treatment-naive patients than in treatment-experienced patients. IP-10 level decreased by approximately 50% in both groups during the first week; in the second week, the decline among treatment-naive patients was substantially greater than that among treatment-experienced patients.

Although patients had a wide distribution of IP-10 levels prior to treatment, the average was approximately 250 pg/mL. Because normal IP-10 levels are approximately 100 pg/mL, the initial 50% reduction achieved with DAA treatment rapidly brought many patients' levels to within normal range. If IP-10 is a measure of how much virus is present in the liver—that is, if it serves as a sensor to monitor live HCV RNA replication complexes—then these findings indicate an extremely rapid decline of these complexes, suggesting that potent treatment with DAAs may actually be eradicating HCV replication complexes from the liver cells. The more rapid second-phase decay of HCV infection observed following potent treatment with DAAs may therefore represent this clearance of HCV replication complexes rather than the slower kinetics expected if this phase of the decay merely reflected the turnover of HCV-infected cells.

Effect of Treatment on the Equilibrium Between HCV and Innate or Adaptive Immunity

HCV infection induces a robust interferon-stimulated gene (ISG) profile, and IL28B and HCV stimulate interferon signaling pathways in hepatocytes in vitro. In patients, exogenous alpha-interferon binds to interferon cell receptors that, through the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, induce ISGs and promote production of a cascade of effectors that can shut down viral replication within the cell. HCV induces lambda-interferon, which engages lambda-interferon receptors and, also through the JAK-STAT pathway, stimulates alpha-interferon production.

In basic terms, within an infected cell, HCV is rapidly uncoated and situated in the endoplasmic reticulum. HCV RNA is exposed, and sequences in the 3′ end of the RNA are recognized by pattern recognition receptors that stimulate the retinoic acid–inducible gene (RIG) I (RIG-I) pathway. This activates the host's innate immune response, including production of alpha- and beta-interferons, and clears the virus from the cell. These responses also protect adjacent cells from infection.

However, it is now known that the HCV nonstructural protein (NS) 3/4A (NS3/4A) protease cleaves mitochondrial antiviral-signaling protein (MAVS; also called virus-induced signaling adapter [VISA]) and Toll/IL-1 receptor domain–containing adaptor inducing beta-interferon (TRIF), 2 adaptor molecules essential for interferon signaling activation, inhibiting RIG-I, and shutting down the downstream cascade that results in activation of the innate immune response, including production of type 1 interferons.[12] In addition to this crucial component of the host-virus interaction, there are other mechanisms by which HCV interferes with the host's innate immune response, including inhibition of pattern recognition receptor signaling by NS4B via interaction with stimulator of interferon genes (STING; also called mediator of interferon regulatory factor 3 activation [MITA]) adaptor protein, another molecule that mediates HCV-induced interferon signaling and inhibits the activation of interferon regulatory factor 3 genes; interference with interferon signaling via inhibition of ISG expression by the viral core; and antagonism of ISGs by the HCV NS5A and envelope (E) 2 (E2) proteins.[13,14] In essence, once it enters the cell, the virus acts to undermine innate immune response using viral enzymes and core and envelope proteins.

In this context, the use of potent DAAs that very rapidly inhibit viral replication also results in rapid elimination of those viral products active in evading the innate immune response. In addition to inhibiting viral replication and destabilizing HCV replication complexes, DAAs may allow for restoration of innate immune activity against HCV, which further primes adaptive immune responses, including production of cytotoxic T cells and virus-specific, neutralizing antibodies. The rapid and profound reduction in HCV replication induced by DAAs may also undermine viral mechanisms that permit evasion of innate and adaptive immune responses and thereby result in a net effect on viral replication that is more than the sum of the parts of the regimen itself.

Conclusion

Remarkable improvements in the treatment of HCV infection have been observed since the availability of HCV DAAs. Evidence is emerging that with increasingly potent and sustained antiviral pressure, the sum of the anti-HCV effect is greater than its parts. The inhibition of viral replication with these drugs results in destabilization of HCV replication complexes, permits cure of infected cells, and promotes the activity of the innate immune response axis in shutting down viral replication by abrogating HCV immune evasion mechanisms.

DAA-containing therapy provides an outstanding platform from which to study virus-host interaction. This will have important implications not just for HCV but for other flaviviruses, such as West Nile virus, dengue virus, and yellow fever virus, that exhibit many of the same mechanisms of immune evasion.

Presented by Dr Schooley in June 2013. First draft prepared from transcripts by Matthew Stenger. Reviewed and edited by Dr Schooley in December 2013.

Financial Affiliations: Dr Schooley has received research support from Bristol-Myers Squibb and Boehringer Ingelheim Pharmaceuticals, Inc, and has served as a scientific advisor to CytoDyn and Merck & Co, Inc, as a scientific advisory board member for Gilead Sciences, Inc, GlobeImmune, Inc, and Monogram Biosciences, and as a member of data monitoring committees for Axio and Gilead Sciences, Inc. He has stock in GlobeImmune, Inc.

References

1. Gulick RM, Mellors JW, Havlir D, et al. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N Engl J Med.* 1997;337:734-739.
2. Zeuzem S, Herrmann E, Lee JH, et al. Viral kinetics in patients with chronic hepatitis C treated with standard or peginterferon alpha2a. *Gastroenterology.* 2001; 120(6):1438-1447.
3. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature.* 2009;461(7265):798-801.
4. Osburn WO, Levine JS, Chattergoon MA, Thomas DL, Cox AL. Anti-inflammatory cytokines, pro-fibrogenic chemokines and persistence of acute HCV infection. *J Viral Hepat.* 2013;20(6):404-413.
5. Rong L, Perelson AS. Treatment of hepatitis C virus infection with interferon and small molecule direct antivirals: viral kinetics and modeling. *Crit Rev Immunol.* 2010;30(2):131-148.
6. Guedj J, Perelson AS. Second-phase hepatitis C virus RNA decline during telaprevir-based therapy increases with drug effectiveness: implications for treatment duration. *Hepatology.* 2011;53(6):1801-1808.
7. Askarieh G, Alsio A, Pugnale P, et al. Systemic and intrahepatic interferon-gamma-inducible protein 10 kDa predicts the first-phase decline in hepatitis C virus RNA and overall viral response to therapy in chronic hepatitis C. *Hepatology.* 2010;51(5):1523-1530.
8. Diago M, Castellano G, Garcia-Samaniego J, et al. Association of pretreatment serum interferon gamma inducible protein 10 levels with sustained virological response to peginterferon plus ribavirin therapy in genotype 1 infected patients with chronic hepatitis C. *Gut.* 2006;55(3):374-379.
9. Lagging M, Romero AI, Westin J, et al. IP-10 predicts viral response and therapeutic outcome in difficult-to-treat patients with HCV genotype 1 infection. *Hepatology.* 2006;44(6):1617-1625.
10. Reiberger T, Aberle JH, Kundi M, et al. IP-10 correlates with hepatitis C viral load, hepatic inflammation and fibrosis and predicts hepatitis C virus relapse or non-response in HIV-HCV coinfection. *Antivir Ther.* 2008;13(8):969-976.
11. Lin JC, Afdhal N, Lawitz EJ, et al. Changes in interferon-γ inducible protein-10 in treatment-naive versus treatment-experienced patients given an all-oral anti-HCV regimen. Poster presented at: 48th Annual Meeting of the European Association for the Study of the Liver (EASL). April 24–28, 2013; Amsterdam, The Netherlands.
12. Liu HM, Gale M. Hepatitis C virus evasion from RIG-I-dependent hepatic innate immunity. *Gastroenterol Res Pract.* 2010;2010:548390.
13. Horner SM, Gale M, Jr. Intracellular innate immune cascades and interferon defenses that control hepatitis C virus. *J Interferon Cytokine Res.* 2009;29(9):489-498.
14. Ding Q, Cao X, Lu J, et al. Hepatitis C virus NS4B blocks the interaction of STING and TBK1 to evade host innate immunity. *J Hepatol.* 2013;59(1):52-58.

Top Antivir Med. 2014;21(5):148-151