HCV IN 2015

Advances in hepatitis C research and treatment

Barbara Rehermann

In 2015, new treatment regimens were revealed that achieve >95% cure rates for all HCV genotypes. The HCV polymerase structure was solved in catalytically relevant HCV replication steps and in the context of nucleotide analogue inhibition. Moreover, HCV research taught us new links between innate antiviral responses, lipid metabolism and intracellular membrane formation.

HCV is an enveloped, positive-stranded RNA virus representing the *Hepacivirus* genus in the Flaviviridae family. More than 185 million people worldwide are chronically infected with HCV and at risk of developing liver cirrhosis and hepatocellular carcinoma[1]. HCV exists in six major genotypes and >100 subtypes and, in each patient, creates an astounding number of quasispecies. This sequence diversity is facilitated by the high virus production rate of more than 10^{12} virions per patient per day and the lack of a proofreading ability of the RNA-dependent HCV polymerase (RdRp).

The year 2015 provided insights into structural changes of RdRp during HCV genome replication and into the structural basis for the antiviral effect of the nucleotide analogue inhibitor (the active form of sofosbuvir) that has revolutionized HCV treatment. The RdRp (encoded by *NS5B*) catalyzes the synthesis of a negative-stranded RNA intermediate that then serves as template for the synthesis of multiple positive RNA strands. The latter are translated into HCV proteins, used as templates for further minus-strand RNA synthesis or are packaged into virions that are released from cells. In an elegant study, Appleby and coauthors[2] stalled the RdRp in two catalytically relevant formations in ternary complexes with the RNA template, RNA primer, incoming nucleotides and Mn^{2+}. The first formation reflects the primed initiation assembly in which viral RNA has already entered the active site of RdRp and a dinucleotide molecule (that later serves as a primer) has been formed complementary to the HCV RNA 3′ end. Compared with the closed apo state of RdRp, the β-loop and C-terminal membrane-anchoring linker are retracted from the active site in this configuration. The subsequent primer-extension activity of the RdRp opens its active site cavity further. From there, the enzyme shifts into an open configuration in which the β-loop and C-terminus or the RdRp are completely moved out of the RNA binding groove, thus allowing elongation of the RNA strand. Co-crystal structures of RdRp and 2′-F′-2′-*C*-methyluridine monophosphate (the active form of sofosbuvir) show recognition and successful incorporation of this 2′ modified nucleotide inhibitor into the growing RNA strand. The incorporation of the active form of sofosbuvir impairs the generation of hydrogen bond networks, prevents conformational changes of the RdRp and promotes RNA chain disruption.

Also in 2015, unprecedented progress in the treatment of HCV infection was seen. The combination of sofosbuvir with the NS5A inhibitor velpatasvir in a 12-week once daily all-oral regimen achieved a 99% sustained virologic response (SVR) in patients with HCV genotype 1, 2, 4, 5, or 6 infection, including those who were previously treated or had compensated cirrhosis[3,4]. The SVR of patients infected with the difficult-to-treat HCV genotype 3 was only slightly lower (95% in previously untreated patients, 91% in patients with previous treatment failure and 89% in those with compensated cirrhosis)[4]. The addition of ribavirin to the regimen resulted in a 94% SVR even in patients with decompensated cirrhosis[5]. With FDA approval expected in 2016, this treatment regimen will be the most successful to date, basically eliminating the category of 'difficult-to-treat' populations.

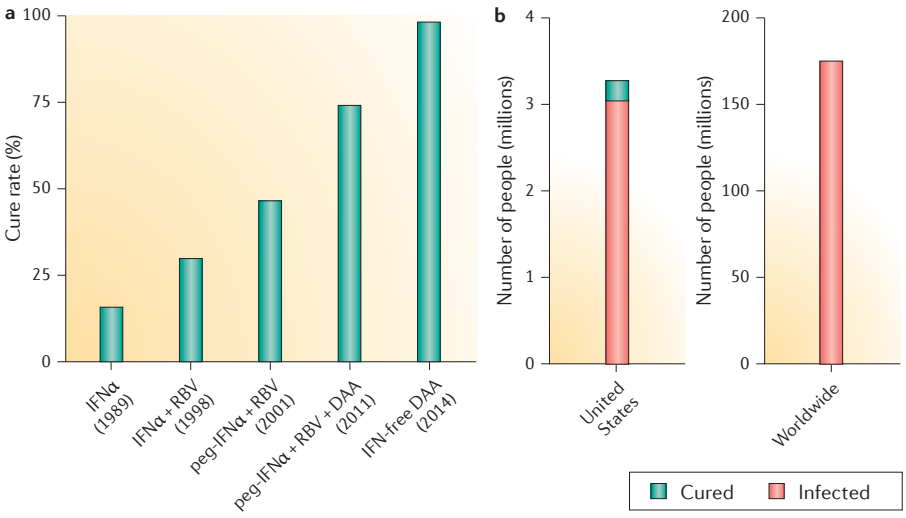

Figure 1 | **Patients cured of HCV infection. a** | Graph shows cured patients as percentage of patients treated with the indicated regimens. Year in brackets indicates when the treatment was introduced. **b** | Absolute number of patients in the USA and worldwide who were cured by 2013 (REF. 6). About 600,000 patients worldwide were treated with sofosbuvir-containing regimens by the end of 2015. Of the 185 million people worldwide infected with HCV, only a minority have been diagnosed. Green bars represent patients infected with HCV and orange bars represent cured patients. Cure is defined as achieving sustained virologic response. DAA, direct acting antivirals; RBV, ribavirin.

Key advances

- The structure of the RNA-dependent RNA polymerase is solved in two catalytically relevant formations during HCV replication, revealing the structural basis for the antiviral effect of sofosbuvir[2]
- The all-oral, interferon-free combination treatment of nucleotide analogue inhibitor sofosbuvir and the NS5A inhibitor velpatasvir achieves 99% sustained virologic response rates in patients with chronic HCV genotype 1, 2, 4, 5, or 6 infection[3]
- Successful HCV treatment is associated with a rapid normalization of intrahepatic inflammation and innate immune cell activation[7]
- The cytosolic lipid-binding protein SEC14-like protein 2 is required for replication of clinical isolates of HCV of diverse genotypes in cell culture[9]
- Increased 25-hydroxysterol levels during HCV infection induce microRNA-185 to regulate lipid metabolism, revealing a new immune–metabolomic host response axis with antiviral function[10]

Given these treatment response rates what remains to be done? Although cure rates have progressively increased in treated patients, it should not be forgotten that the total number of treated patients still remains small[1,6] (FIG. 1). The large number of undiagnosed HCV infections, the high costs of the new treatment regimens and the limited access of high-risk populations to treatment make it unlikely that the global HCV reservoir will be eliminated in the foreseeable future. This reality has re-emphasized the need for a prophylactic vaccine[1].

For those who are fortunate to have access to treatment, further studies that assess the continued risk of cirrhosis and hepatocellular carcinoma will be important. Although a rapid normalization (to levels seen in healthy individuals) of biomarkers of intrahepatic inflammation and natural killer cell activation, within 8 weeks of successful therapy has been reported in 2015 (REF. 7), the extent and speed with which advanced liver fibrosis and cirrhosis resolves is still unknown. In addition, basic studies on the pathogenesis of HCV-induced liver disease are still valuable because HCV infection continues to teach us many new aspects of intracellular innate immunity and cell biology. This point is exemplified by the following studies published this year.

HCV manipulates the intracellular environment to either co-opt or antagonize host factors. Much of this manipulation is facilitated by the formation of an HCV-induced membranous web that allows HCV replication in a customized, favourable environment enriched in cholesterol and lipids, and protected from host ribonucleases and exonucleases, and to some extent, from innate immune sensors. The membranous web is induced by both viral and host factors and several of the HCV proteins are membrane-associated. However, HCV replication induces oxidative stress with peroxidation of polyunsaturated fatty acids within the membranes web[8]. This damage alters the configuration of membrane-anchored HCV proteins and limits HCV replication[8]. In a gain-of-function approach Saeed *et al.*[9] have now identified SEC14-like protein 2 (also known as alpha-tocopherol-associated protein) as a critical host factor for HCV replication. SEC14-like protein 2 is a cytosolic lipid binding protein that enhances the accumulation of vitamin E (tocopherol) as well as the vitamin-E-mediated inhibition of lipid peroxidation. Ubiquitously expressed in tissues, SEC14-like protein 2 is absent in hepatoma cell lines. A limited number of HCV tissue culture models exist, and involve specific HCV strains or HCV replicons with cell-culture adaptive mutations. Overexpression of SEC14-like protein 2 in hepatoma cells has now created an *in vitro* model to study 'natural' patient-derived HCV isolates with diverse genotypes[9].

> “...it should not be forgotten that the total number of treated patients still remains small”

Also in 2015, Singravelu and colleagues[10] identified a new immune–metabolomic host response axis with antiviral function that exploits the dependency of HCV on intracellular lipids. Antiviral type I interferon responses induce the interferon-stimulated gene, cholesterol-25-hydoxylase, in macrophages and dendritic cells resulting in increased 25-hydroxysterol levels in the liver and blood of patients infected with HCV. Although 25-hydroxysterol is already known to inhibit the fusion of several viruses to cell membranes, Singaravelu *et al.*[10] identified the 25-hydroxysterol-mediated induction of microRNA (miR)-185 as an additional antiviral effector mechanism. Interestingly, miR-185 does not target the HCV RNA genome directly. Rather, it decreases mRNA levels of host genes that promote lipid uptake, synthesis of triglycerides and cholesterol, and desaturation of fatty acids, thereby affecting membranous web formation and decreasing HCV replication. Using mice with chimeric human livers as a model for HCV infection, the authors showed that HCV tries to counteract this effect by decreasing intracellular levels of miR-185 and miR-130 (the latter is not induced by 25-hydroxysterol, but stimulates the expression of miR-185).

Collectively, these findings exemplify that HCV continues to teach us new insights into intracellular immune responses. Despite being a master teacher of cell biology, HCV also revealed its achilles heel because it can now be eradicated in >95% of treated patients with pan-genotype all-oral treatment regimens. These unprecedented response rates extend to patients with HCV genotype 3 infections and/or decompensated cirrhosis, which were formerly termed 'difficult-to-treat'. Global eradication efforts should now focus on reducing the number of undiagnosed persons and advancing treatment regimens to allow shorter treatment duration, reduce treatment costs and make it more accessible. Finally, a focus on public health measures and vaccine development to reduce HCV spread among populations with high infection and re-infection risk is needed.

Barbara Rehermann is at the Immunology Section, Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, DHHS, 10 Center Drive, Bethesda, Maryland 20892, USA.
rehermann@nih.gov

doi:10.1038/nrgastro.2015.227
Published online 21 Jan 2016

1. Cox, A. L. Global control of hepatitis C virus. *Science* **349**, 790–791 (2015).
2. Appleby, T. C. *et al.* Structural basis for RNA replication by the hepatitis C virus polymerase. *Science* **347**, 771–775 (2015).
3. Feld, J. J. *et al.* Sofosbuvir and velpatasvir for HCV genotype 1, 2, 4, 5, and 6 infection. *N. Engl. J. Med.* http://dx.doi.org/10.1056/NEJMoa1512610.
4. Foster, G. R. *et al.* Sofosbuvir and velpatasvir for HCV genotype 2 and 3 infection. *N. Engl. J. Med.* http://dx.doi.org/10.1056/NEJMoa1512612.
5. Curry, M. P. *et al.* Sofosbuvir and velpatasvir for HCV in patients with decompensated cirrhosis. *N. Engl. J. Med.* http://dx.doi.org/10.1056/NEJMoa1512614.
6. Holmberg S. D. *et al.* Hepatitis C in the United States. *N. Engl. J. Med.* **368**, 1859–1861 (2013).
7. Serti, E. *et al.* Successful interferon-free therapy of chronic hepatitis C virus infection normalizes natural killer cell function. *Gastroenterology* **149**, 190–200.e2 (2015).
8. Yamane, D. *et al.* Regulation of the hepatitis C virus RNA replicase by endogenous lipid peroxidation. *Nat. Med.* **20**, 927–935 (2014).
9. Saeed, M. *et al. SEC14L2* enables pan-genotype HCV replication in cell culture. *Nature* **524**, 471–475 (2015).
10. Singaravelu, R. *et al.* MicroRNAs regulate the immunometabolic response to viral infection in the liver. *Nat. Chem. Biol.* **11**, 988–993 (2015).

Acknowledgements
This work was supported by the Intramural Research Program of the NIH, The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

Competing interests statement
The author declares no competing interests.