

NEW HORIZONS

Immunological Aspects of Antiviral Therapy of Chronic Hepatitis B Virus and Hepatitis C Virus Infections

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Hepatitis B virus (HBV) and hepatitis C virus (HCV) cause a large proportion of acute and chronic liver disease worldwide. Over the past decades many immunological studies defined host immune responses that mediate spontaneous clearance of acute HBV and HCV infection. However, host immune responses are also relevant in the context of treatment-induced clearance of chronic HBV and HCV infection. First, the pretreatment level of interferon-stimulated genes as well as genetic determinants of innate immune responses, such as single nucleotide polymorphisms near the *IFNL3* gene, are strong predictors of the response to interferon-alpha (IFN- α)-based therapy. Second, IFN- α , which has been a mainstay of HBV and HCV therapy over decades, and ribavirin, which has also been included in interferon-free direct antiviral therapy for HCV, modulate host immune responses. Third, both IFN- α -based and IFN- α -free treatment regimens of HBV and HCV infection alter the short-term and long-term adaptive immune response against these viruses. Finally, treatment studies have not just improved the clinical outcomes, but also provided opportunities to study virus-host interaction. This review summarizes our current knowledge on how a patient's immune response affects the treatment outcome of HBV and HCV infection and how innate and adaptive immune responses themselves are altered by the different treatment regimens. (HEPATOLOGY 2015;61:712-721)

Spontaneous hepatitis B virus (HBV) and hepatitis C virus (HCV) clearance occurs most frequently in the acute phase of infection and is mediated by vigorous adaptive immune responses. In contrast, spontaneous viral clearance rarely occurs in the chronic phase of HBV infection and almost never in the chronic phase of HCV infection when virus-specific T-cell responses are exhausted. While host immune responses that result in spontaneous HBV and HCV clearance have been extensively characterized, it has recently become clear that they also play a role in the context of antiviral therapy in chronic infection (Table 1).

Immunological Aspects of HBV Antiviral Therapy

When assessing the prospects of successful antiviral therapy of chronic HBV infection one has to consider

that even spontaneously recovered patients do not completely clear HBV and may reactivate the infection upon immunosuppression, e.g., during cancer chemotherapy or organ transplantation.¹ Spontaneous recovery with seroconversion to hepatitis B virus surface antigen (HBsAg)-negative/anti-HBs-positive is observed in >95% of acute HBV infections and <1%/year of chronic HBV infection. Although spontaneous recovery results in lifelong protective immunity, trace amounts of HBV DNA appear sporadically in the circulation.² These trace amounts of HBV can be infectious and stimulate HBV-specific antibody and T-cell responses, which in turn control viremia. Thus, natural immunity is considered protective rather than sterilizing. This is because the transcriptional template of the virus, the covalently closed circular DNA (cccDNA), persists in the form of a minichromosome in host cells.³ Immunosuppression, e.g., as part of cancer chemotherapy or

Abbreviations: cccDNA, covalently closed circular DNA; HBV, hepatitis B virus; HCV, hepatitis C virus; IFN- α , interferon-alpha; ISG, IFN-stimulated gene; PBMC, peripheral blood mononuclear cells; SOCS3, suppression of cytokine signaling 3; WHV, woodchuck hepatitis virus.

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Table 1. Comparison of Clinical, Virological, and Immunological Features of HBV and HCV Infection

	HBV	HCV
Chronic infections	350 million people infected	170 million people infected
Cause of chronic infection	Mostly vertical/perinatal transmission: mother-to-neonate transmission most common worldwide, followed by childhood infection	Mostly horizontal transmission: injection drug use, parenteral, sexual, nosocomial
Virology		
Virus	42 nm; enveloped nucleocapsid; partially double-stranded DNA genome	50 nm; enveloped nucleocapsid; positive stranded RNA genome
Family	Hepadnaviridae	Flaviviridae; Hepacivirus genus
Genotypes	8 genotypes	6 major genotypes; more than 50 subtypes; quasispecies in each infected patient
Mutation rate	Low	High
Virus half-life	2-3 days	3 hours
Virus production	10^{10} - 10^{12} virions/day	10^{12} virions/day
Natural immunity		
Innate response	Minimal IFN and ISG response	Strong IFN and ISG response
Protective response		
- primary infection	T cells, neutralizing antibodies	T cells; transient strain-specific neutralizing antibodies?
- secondary infection	T cells, neutralizing antibodies	T cells
Virus clearance	No; cccDNA (transcriptional template) may persist; disease reactivation possible	Yes
Therapy of chronic infection		
Targets	HBV replication HBV transcription (cccDNA)	HCV replication
IFN- α based therapy	- Cure (seroconversion) possible but rare; - Induces epigenetic changes in cccDNA; - Slow decrease in viral titer; - ISG and NK cell activation - Suppression of HBV-spec. T cells	Cure (SVR) possible - Fast decrease in viremia; - ISG and NK cell activation in inverse correlation to pretreatment levels (genetically determined); - Suppression of HCV-spec. T cells
IFN-free antiviral therapy	- Cure (seroconversion) very unlikely; - Fast reduction in viremia - Partial reversal of T-cell exhaustion	- High incidence of cure (SVR) - Decreased ISG expression - Fast reduction in viremia - Partial reversal of T-cell exhaustion

Abbreviations: IFN, interferon; ISG, interferon-stimulated gene; NK cell, natural killer cell; SVR, sustained virological response.

organ transplantation, carries the risk of HBV reactivation.¹ An ideal antiviral therapy should therefore not just decrease HBV replication but also eliminate or at least control cccDNA. Antiviral therapy may need to synergize with and ideally boost adaptive immune responses to achieve this.

Interferon (IFN)- α -Based Therapy. The rationale for using IFN- α to treat chronic HBV infection is identical to its use in chronic HCV infection: IFN- α exerts direct antiviral effects and induces complex

immune alterations that ideally result in the clearance of the respective viruses.

In a small percentage of patients with chronic HBV infection IFN- α -based therapy results in HBeAg and HBsAg loss, seroconversion to anti-HBe and anti-HBs status, reduction of viral replication, and alanine aminotransferase (ALT) normalization.⁴ High doses of IFN- α have been shown to induce the degradation of HBV cccDNA in cell culture models by up-regulating the expression of the deaminase APOBEC3A and to

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deaminate cccDNA,⁵ presumably when it is transiently rendered single-stranded by RNA polymerase II before transcription initiation. Cytidine deamination results in apurinic/apyrimidinic site formation and in degradation of cccDNA degradation by endonucleases.⁵ IFN- α also induces epigenetic changes in cccDNA-bound histones, which results in inhibition of HBV replication and decreases the transcription of pregenomic RNA and subgenomic RNA from cccDNA.^{6,7} Finally, IFN- α accelerates decay of nucleocapsids that contain pregenomic HBV RNA.^{8,9} The direct antiviral effect of IFN- α is sufficient to induce a reduction in viral load and HBV antigen levels as shown in HBV-infected humanized uPA/SCID mice that lack immune cells.¹⁰

Successful antiviral treatment also appears to synergize with the natural trend to viral control in some patient populations, such as HBeAg+ patients with increased aminotransferase levels. These patients are not only more likely to establish partial immune control of HBV replication with seroconversion to HBeAg-negative/anti-HBeAg-positive status and a decrease in disease activity, but are also more likely to respond to antiviral therapy.

Unfortunately, both the induction of endogenous IFN- α during the natural course of HBV infection and the antiviral function of exogenously administered IFN- α are not fully effective in most patients. Patients with acute HBV infection or HBV reactivation do not have much evidence of IFN- α production,¹¹ and IFN-stimulated genes (ISGs) are neither significantly induced in the livers of chimpanzees with acute HBV infection¹² nor in the livers of woodchucks chronically infected with woodchuck hepatitis virus (WHV), which is closely related to HBV.¹³ The reasons for HBV's ability to avoid a robust IFN- α -mediated innate response are controversial and have been reviewed in detail elsewhere.¹⁴ In addition to hiding from intracellular innate immune mechanism of pathogen detection,¹⁵ HBV may prevent Jak STAT signaling.¹⁶ To explain the overall low antiviral effect of IFN- α on HBV-infected hepatocytes, a recent report has suggested that its antiviral activity is mediated by exosomes that are secreted by noninfected nonparenchymal cells.¹⁷

The limited antiviral effects of IFN- α in HBV infection are reflected by the relatively small percentage of patients with chronic HBV infection who respond to IFN- α -based therapy with HBeAg and HBsAg loss, seroconversion to anti-HBe and anti-HBs status, reduction of viral replication, and ALT normalization.⁴ A decrease in HBV viremia is observed about 3-4 weeks after the start of IFN- α -based therapy^{18,19} and takes several months to reach its maximum,²⁰ whereas a sharp decrease in HCV viremia occurs within the first 48 hours of IFN- α -based therapy.²¹

This delayed kinetic may reflect a progressive recovery of immune-mediated endogenous antiviral mechanisms, which, however, have scarcely been analyzed. A recent study characterized innate and adaptive immune responses of HBeAg-negative HBV-infected patients during 48 weeks of pegylated (Peg)IFN- α therapy.²⁰ The decrease in HBV viremia coincided with an increase in the frequency of circulating CD56^{bright} natural killer (NK) cells, increased expression of the activating receptor NKp46, and the cytotoxic receptor TRAIL by NK cells and recovery of their IFN- γ production.²⁰ The improved function of CD56^{bright} NK cells paralleled an increase in IL-15 levels, which may have stimulated the NK cells.²⁰ These observations were subsequently extended to HBeAg-positive patients.²² Interrogation of the transcriptome of peripheral blood mononuclear cells (PBMC) showed ISG induction within 6 hours after the start of therapy. This study also confirmed the ability of IFN- α treatment to boost IL-15 (in addition to IL-6 and CXCL-10) levels and to trigger NK cell proliferation. However, the robust activation of innate immune parameters was not associated with a decrease in HBV titer within the first 2 weeks of therapy, suggesting that the modulation of innate immune responses was a direct effect of the administered IFN- α and not secondary to its antiviral effect.

In contrast to innate immune responses, HBV-specific T-cell responses did not recover during IFN-based therapy.^{20,23} This is unexpected because IFN- α is known to increase T-cell survival,²⁴ expression of antigen-processing enzymes,²⁵ cross-presentation of T-cell antigens,²⁶ and production of interleukin (IL)-12,²⁷ a cytokine that has been shown to rescue the function of exhausted HBV-specific T cells *in vitro*.²⁸ While the frequency of activated T cells increases within the first 2-3 days of IFN- α -based therapy, a numeric reduction in CD8 T cells, in particular late effector CD8 T cells, is observed during long-term treatment.²⁰ Moreover, the HBV-specific CD8 T-cell subset maintains expression of inhibitory cell surface markers such as PD-1, Lag-3, and CTLA-4,²² and thus remains nonfunctional throughout therapy.²⁰ Similar negative effects of IFN- α on virus-specific adaptive immunity have recently been emphasized in two studies of mice that were chronically infected with lymphocytic choriomeningitis virus. In these studies, T-cell responses were restored by inhibiting the effects of virus-induced IFN- α rather than by treating the infection with IFN- α .^{29,30} Although this scenario is not fully applicable to HBV-infected patients, where IFN- α signaling is suppressed,^{13,31} the strong and continued activation of ISGs during IFN- α -based

therapy²² may negatively impact the function of HBV-specific T cells. Accordingly, flares in liver disease activity are often observed after cessation of IFN-based therapy. Seroconversion to anti-HBe may follow a flare and is specifically associated with an increase in IL-12, IFN- γ , and IL-2 serum levels.³² While a recovery of CD8 T-cell function is not observed during IFN-based therapy,²² cytotoxic CD8 T-cell lines can be more readily expanded from the blood of patients who have responded to IFN-based therapy with anti-HBe or with anti-HBe/anti-HBs seroconversion than from nonresponders³³

Thus, IFN- α -based therapy stimulates predominantly innate immune responses. A note of caution is warranted because all published data are based on interrogation of immune responses in the blood. Although reduced expression of ISGs in the liver of chronic HBV patients mirrors that in PBMCs,³¹ the intrahepatic microenvironment poses additional limitations to the effector arm of antiviral immunity. For example, intrahepatic levels of suppression of cytokine signaling 3 (SOCS3), a negative regulator of cytokine signaling, are increased in patients with chronic HBV infection and in woodchucks with chronic WHV infection.^{13,31} SOCS3 has not only been associated with HBV's or WHV's ability to evade innate immunity but it is a predictor of poor IFN- α responses in HCV-infected patients³⁴ and as such may also impact the therapeutic efficacy of IFN- α in patients with chronic HBV infection. Because the intrahepatic environment in chronic HBV infection is also rich in IL-10,³⁵ transforming growth factor beta (TGF- β),³⁶ and arginase,³⁷ factors that impair both T and NK cell function, it may not be surprising that the activation of innate immune cells within the first hours of IFN- α treatment is not accompanied by a reduction in HBV titer. A decrease in intrahepatic inflammation, as observed in chronic HBV patients who are treated with NUCs, may be required to restore immune responses in the liver.

Therapy With Nucleos(t)ide Analogs (NUC). HBV-DNA titers decline rapidly in blood and liver during treatment with NUCs, and inflammatory liver disease improves. The potent antiviral effect of NUCs is based on an inhibition of the reverse transcriptase function of the HBV DNA polymerase, the enzyme responsible for HBV replication.³⁸ The rapid reduction of viral replication should theoretically blunt any potential immunomodulatory effect of HBV and restore IFN- α signaling in infected hepatocytes.³⁹ This hypothesis is supported by the recent description of a progressive expression of ISGs in PBMCs of HBeAg+ HBV-infected patients as early as day 8 of treatment

with tenofovir along with a 100-fold decrease in HBV titer.²²

The impact of NUC treatment on innate immune cells is controversial. While a recovery of the function of myeloid dendritic cells has been observed in adefovir-treated patients,⁴⁰ the effect of NUCs on NK cells is less clear. An improvement of NK cell IFN- γ production has been described in a study of entecavir-treated patients,⁴¹ but other studies of adefovir and lamivudine-treated patients did not confirm these results and instead showed decreased expression of the NK cell effector molecule TRAIL.³⁵ Since TRAIL-mediated hepatocyte lysis is thought to contribute to liver injury and inflammation in chronic HBV infection, the latter finding would be consistent with the decrease in liver inflammation that is characteristic for treatment with NUCs.

The reported effects of NUC treatment on adaptive immune cells are more consistent, because an increased frequency and function of HBV-specific CD4 and CD8 T cells is reported in multiple studies.⁴²⁻⁴⁹ Telbivudine and lamivudine both reduce the expression of the inhibitory molecule PD-1 on total T cells and their HBV-specific subset,^{46,47} and telbivudine has also been associated with improved production of IL-21 and recovery of follicular helper T cells, which provide functional help to both T and B cells.⁵⁰ However, this recovery of T-cell function remains incomplete and quantitatively not comparable to adaptive immune responses of patients who spontaneously control HBV infection. It is mainly detectable with assays that are based on *in vitro* expansion of T cells and not readily observed with *ex vivo* assays.⁵¹ Furthermore, restoration of HBV-specific T-cell responses can be transient, which may contribute to the frequent failure of HBV control when treatment is discontinued.^{43,52} Likewise, NUC-mediated HBV suppression is not sufficient to restore responsiveness to HBsAg vaccination⁵³ and lamivudine/IL-12 combination therapy is equally ineffective in boosting HBV-specific T-cell responses.⁵⁴

While a consensus exists on the ability of NUC therapy to partially recover HBV-specific T-cell function, the underlying mechanisms are controversial. It has been suggested that a substantial decline of HBV antigen levels in the blood is required for recovery of T-cell function, because high HBV antigen levels are regarded as the cause of HBV-specific T-cell deletion and exhaustion in natural infection.¹⁴ Unfortunately, with the exception of very few patients who clear HBsAg during treatment,⁴⁹ NUCs have little impact on HBV antigen levels,⁵⁵ particularly in the first year of therapy when HBV-specific T-cell recovery is more

pronounced. As an alternative hypothesis it has been proposed that decreased HBV replication by limiting HSP60 release from infected hepatocytes, results in a secondary decrease in the frequency of CD4 T cells with regulatory/suppressor capacity (Tregs).⁵⁶ According to that hypothesis, NUC-mediated inhibition of HBV replication and reduction in Treg frequency would enhance the HBV-specific T-cell response.⁵⁷ However, even though a reduced frequency of circulating Treg cells was observed in entecavir-, adefovir-, and tenofovir-treated patients,^{56,58,59} it is not clear whether this is the result of reduced HBV replication or reduced liver inflammation.

In this context, it is surprising that a direct immunomodulatory effect of NUCs has not been contemplated in HBV infection. Tenofovir and adefovir have been shown to increase the production of TNF- α , IL-6, IL-10, CCL5 (Rantes), and CCR5 (MIP-1 α) in human monocytes.⁶⁰ Tenofovir has also been shown to modulate Toll-like receptor (TLR)-mediated responses and to reduce the threshold of T-cell activation.⁶¹ A better evaluation of these direct immune effects is attractive because further attempts to boost HBV-specific immune responses will be performed in patients in whom HBV replication is suppressed by some of the potent NUCs. Along this line, it has been shown that entecavir enhances the effects of therapeutic vaccination in the woodchuck model of chronic hepatitis.⁶²

IFN- α /NUC Combination Therapy. The limited efficacy of IFN- α or NUC monotherapy in achieving HBsAg seroconversion and/or sustained HBV control was the rationale for studies that combine the two classes of drugs. Because NUCs potentially inhibit HBV replication and partially restore HBV-specific T-cell responses, and because IFN- α targets HBV cccDNA and stimulates NK cell responses, the combination of IFN- α and NUCs may generate a better antiviral response and immune recovery in patients with chronic HBV infection. Combination NUC/PegIFN- α therapy does indeed have a slightly better on-treatment antiviral effect in HBeAg+ and in HBeAg- patients than IFN- α or NUC monotherapy, but the rate of sustained off-treatment responses is not higher than with PegIFN- α monotherapy.⁶³⁻⁶⁸ A longitudinal study of lamivudine and PegIFN- α -treated children with infancy-acquired HBV infection, a patient group that is considered immunotolerant, demonstrated a recovery of HBV-specific T-cell responses in those who responded with HBsAg loss.⁶⁹ The magnitude of the recovered T-cell response was, however, weak and a comparison with children treated with lamivudine

monotherapy was not performed. Based on recent data, an extended period of viral suppression prior to NUC/PegIFN- α combination therapy may increase the HBs seroconversion rate, as evidenced by HBsAg loss in 60% of patients who were treated with PegIFN and entecavir after more than 3 years of complete viral suppression by entecavir monotherapy.⁷⁰

Immunological Aspects of HCV Antiviral Therapy

In contrast to HBV, HCV can be completely cleared by an individual's immune response. Key mediators of spontaneous HCV clearance are virus-specific T cells (reviewed in⁷¹), which remain readily detectable in the circulation for decades after clearance.⁷² Studies in chimpanzees were important to demonstrate that these T cells protect upon reinfection because depletion of either CD4 or CD8 T cells after recovery from a primary challenge abrogates protective immunity upon HCV rechallenge.^{73,74} Based on the fact that acute HCV infection can be cleared spontaneously, effective antiviral therapy of chronic infection should be able to completely eliminate HCV without the risk of relapse. This is supported by the experience that a sustained virological response (SVR) after IFN-based therapy is durable in more than 97% of patients.⁷⁵

Over the past three decades, treatment regimens have evolved from IFN- α to PegIFN- α /ribavirin combination therapy,⁷⁶ to inclusion of the first protease inhibitors and, most recently, to potent interferon-free combinations of two and three direct-acting antivirals.^{77,78} In addition, the use of IFN- α has stimulated virological and immunological studies on host responses to endogenous and therapeutic IFN- α . This line of research has, for example, led to the discovery of genetic determinants of IFN-responsiveness⁷⁹⁻⁸² and to the identification and characterization of key molecules in antiviral defense, such as the signaling molecule MAVS⁸³ and a broad spectrum of antiviral IFN-stimulated genes.⁸⁴

IFN- α -Based Therapy. IFN- α was used to treat patients with chronic HCV infection, then known as non-A non-B hepatitis, years prior to the identification of the virus itself.⁸⁵ Treatment outcomes improved significantly after the nucleoside analog ribavirin was added to prevent relapse⁸⁶ and again, after IFN- α 's pharmacokinetics was optimized by pegylation (PegIFN- α).⁸⁷ Treatment responders typically exhibit a 1-2 log₁₀ decline in viremia within 1-2 days of treatment initiation, a response that is, as discussed, much faster than that observed in HBV-infected patients.

This first-phase response is thought to be due to inhibition of HCV replication and is followed by a slower second-phase response.²¹ The overall response to therapy is determined by viral factors such as viral titer and genotype and by host factors, which include age, race, gender, cirrhosis, comorbidities, and the baseline activation level of the endogenous IFN- α response.

IFN- α -based therapy of acute HCV infection is highly successful, with >90% of patients responding.⁸⁸ This may be due to the fact that a type II IFN signature prevails at the time of maximal intrahepatic T-cell responses in acute infection (due to IFN- γ production by HCV-specific T cells) and that type I IFN-induced negative feedback and resistance mechanisms are not yet fully induced.⁸⁹ In contrast, IFN- α -based therapy has much lower response rates in chronic HCV infection. High pretreatment levels of type I and type III interferon induced genes and the presence of the unfavorable rs12979860-T allele in the *IFNL3* gene locus⁷⁹⁻⁸¹ are associated with treatment nonresponse. Presence of the Δ G allele of the ss469415590 variant (*IFNL4*- Δ G) adds negative predictive value in African Americans.⁸² While the molecular mechanisms that underlie these associations require further investigation, it has become clear that high baseline ISG levels limit further induction of these genes during IFN- α -based therapy. Induction of negative feedback mechanisms, such as SOCS likely contributes to this refractoriness.⁹⁰

A patient's responsiveness to IFN- α -based therapy can also be assessed by the activation of innate immune cells. Similar to what is observed during IFN- α -based therapy of chronic hepatitis B,²⁰ NK cells are strongly activated within hours of IFN- α injection with increased cytotoxicity and decreased IFN- γ production.⁹¹ This response pattern is due to IFN- α -mediated induction of STAT1 in NK cells, which displaces STAT4 at the IFN- α/β receptor.⁹² Continued IFN- α stimulation then results in preferential STAT1 over STAT4 phosphorylation, with an increase in pSTAT1-dependent NK cell cytotoxicity and a decrease in pSTAT4-dependent IFN- γ production. Accordingly, NK cells from patients with a rapid first phase HCV RNA decline exhibit maximal responsiveness to type I IFN as evidenced by a high induction of pSTAT1 in NK cells compared to patients with slow HCV RNA decline.⁹³ This is consistent with greater NK cell cytotoxicity in treatment responders than nonresponders.⁹¹

The negative correlation between pretreatment and on-treatment activation of IFN- α -responsive immune cells such as NK cells recapitulates what has been reported for intracellular ISGs in hepatocytes. For

example, the expression levels of the activating NK cell receptors NKp30, NKp46, and DNAM-1 correlate inversely with the increase in these parameters during IFN- α -based treatment and the subsequent virological treatment response.⁹⁴ Similar to chronic activation of the innate immune response, chronic activation of the adaptive immune response also predicts treatment failure. This is evidenced by increased pretreatment expression of the programmed death 1 (PD-1) molecule on lymphocytes of treatment nonresponders.⁹⁵ Expression of PD-1 reflects T-cell activation by way of the T-cell receptor,⁹⁶ and PD-1 blockade by itself, is not sufficient to induce HCV clearance in the chimpanzee model.⁹⁷ Longitudinal analysis of HCV-specific T-cell reactivity during IFN-based therapy has shown some differences between those with a fast and slow early response.⁹⁸ Overall, however, IFN- α -based therapy suppresses rather than stimulates HCV-specific T cells. As in chronic HBV infection, the frequency of HCV-specific T cells and their capacity to produce IFN- γ decreases in HCV-infected patients who undergo IFN- α -based therapy.⁹⁹ An exception is treatment during the acute phase of HCV infection. Although the bulk HCV-specific T-cell response as measured by IFN- γ production in response to overlapping HCV peptides also decreases when patients with acute HCV infection are treated,^{100,101} a very small population of HCV-specific memory T cells can be rescued.¹⁰²

Ribavirin. The guanosine analogue ribavirin is a component not only of interferon-based therapies for HCV but also of new therapies with direct-acting antivirals (DAAs). While ribavirin itself only has a modest effect on HCV RNA levels and does not induce viral clearance,¹⁰³ it is associated with a faster second-phase virological response when administered together with PegIFN- α ,¹⁰⁴ and it decreases on-treatment virological breakthrough and posttreatment virological relapse¹⁰⁵ and/or DAAs.¹⁰⁶ Several direct effects on viral replication have been proposed as ribavirin's mechanism of action. These include inhibition of the HCV NS5B-encoded RNA polymerase, depletion of guanosine triphosphates by interference with a host enzyme, and promotion of viral error catastrophe (reviewed in¹⁰⁷). While each of these mechanisms is supported by *in vitro* data, several *in vivo* observations argue against a primarily antiviral mode of action. For example, ribavirin monotherapy results only in a modest, less than a 1 log₁₀ decline in viral levels,¹⁰⁴ does not increase the rate of nucleotide substitutions,¹⁰⁸ and does not improve the first-phase virological decline in combination therapy with PegIFN- α .¹⁰⁹ An immunomodulatory

effect has been proposed because ribavirin induces a set of ISGs that differs from those induced by IFN- α ^{110,111} and may improve the ability of the HCV-infected liver to respond to IFN- α -based therapy.¹¹² Consistent with this proposed synergistic action, ribavirin accelerates the second-phase virological response in patients with a sub-optimal, but not absent, interferon response.¹¹³ In addition, ribavirin counteracts IFN- α 's negative effect on NK cell IFN- γ production to some extent¹¹⁴ and improved IFN- γ production by NK cells correlates with the second-phase virological response.¹¹⁴ Whether it also contributes to better immunosurveillance and prevention of virological relapse is an interesting question for further studies. Finally, ribavirin was shown to enhance T-cell function and to inhibit production of the immunosuppressive cytokine IL-10 *in vitro*¹¹⁵ but this has not been confirmed with T cells from treated patients.

DAA. The development of interferon-free regimens that combine two or three DAAs and achieve high response rates for genotypes 1, 2, and 3 infections^{77,78} is exciting not only from a clinical, but also from an immunological perspective. A key question is whether effective inhibition of HCV replication restores defective innate and adaptive immune responses. Inhibition of HCV replication by interferon-free DAA regimens may decrease the virus-induced ISG signature and restore IFN-responsiveness. It would also be of interest to investigate whether these regimens recover NK cell IFN- γ production, which is suppressed in chronic HCV infection.^{116,117} Finally, it is important to investigate whether exhausted and dysfunctional T-cell responses recover. A recent study demonstrates that both the frequency of HCV-specific CD8 T cells in the blood and their proliferative *in vitro* response increases in DAA responders but not in nonresponders.¹¹⁸ Of note, DAA-mediated viral clearance preferentially increased the frequency of T cells that targeted conserved HCV epitopes—most of these T cells were thought to be exhausted due to chronic antigenic stimulation. While it is not possible to distinguish between the outgrowth of a small number of functional T cells and reversion of T-cell exhaustion, and while it is not yet clear whether increased proliferation is associated with increased effector function, it is important that a functional T-cell response may contribute to prevention of viral relapse and establishment of long-term immunity against reinfection. Future studies may clarify the extent and mechanisms of improved T-cell function. A reversion of T-cell exhaustion may also be relevant for other diseases, such as HIV infection and cancer, where T-cell exhaustion has been described.

Future Directions

For chronic hepatitis B, the current treatment regimens rarely result in complete eradication of the virus and its cccDNA. New regimens are needed that complement antiviral therapy with strategies to induce better immune control. This may be achieved by enhancing immune responses directly (TLR-agonists, vaccine therapy, adoptive T-cell therapy) or indirectly (through inhibition of HBsAg production). For chronic HCV infection, the prospect is different because antiviral therapies can completely eradicate the virus. IFN- α -free regimens that are effective against all HCV genotypes and that can be administered orally with minimal side effects appear within reach. However, even with a reduction in treatment duration and costs, these regimens will likely not be affordable in countries where the prevalence of HCV and the incidence of new infections are highest. The development of a prophylactic vaccine that induces protective immunity is therefore still worth pursuing.

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