REVIEW ARTICLE

Reversing immune dysfunction and liver damage after direct-acting antiviral treatment for hepatitis C

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ABSTRACT

The introduction of small molecules targeting viral functions has caused a paradigm shift in hepatitis C virus (HCV) treatment. Administration of these direct-acting antivirals (DAAs) achieves a complete cure in almost all treated patients with short-duration therapy and minimal side effects. Although this is a major improvement over the previous pegylated interferon plus ribavirin (PEG-IFNα/RBV) standard-of-care treatment for HCV, remaining questions address several aspects of the long-term benefits of DAA therapy. Interferon (IFN)-based treatment with successful outcome was associated with substantial reduction in liver disease–related mortality. However, emerging data suggest a complex picture and several confounding factors that influence the effect of both IFN-based and DAA therapies on immune restoration and limiting liver disease progression. We review current knowledge of restoration of innate and HCV-specific immune responses in DAA-mediated viral elimination in chronic HCV infection, and we identify future research directions to achieve long-term benefits in all cured patients and reduce HCV-related liver disease morbidity and mortality.

KEYWORDS: direct-acting antiviral; hepatitis C; immune dysfunction

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INTRODUCTION

Hepatitis C virus (HCV) infection affects more than 71 million individuals worldwide. Around 25% of acute HCV infections resolve spontaneously, and the remaining 75% develop into chronic infection and progressive liver damage, including fibrosis, cirrhosis, and hepatocellular carcinoma (HCC; reviewed in [1]). For more than a decade, the combination of pegylated interferon alpha and ribavirin (PEG-IFNα/RBV) was the standard-of-care

therapy for chronic HCV. This treatment was moderately effective in achieving a sustained virologic response (SVR) in approximately half of treated individuals (2). Furthermore, interferon (IFN)-based therapy was long (~48 wk) and was associated with substantial side effects (3). Direct-acting antivirals (DAAs) that target and inhibit key HCV life-cycle proteins, including the NS3/4A protease, NS5A, and the RNA polymerase NS5B, have been available since 2011, with new molecules in the approval pipeline (4). Multiple all-oral DAA combinations are pan-genotypic, with SVR rates of nearly 99%, shorter treatment duration (12 wk), and minimal side effects (5,6). Data on the IFN-based therapies demonstrate that SVR is associated with substantial reduction in liver disease–related mortality (7,8). Early but not late IFN therapy is also associated with restoration of HCV-specific immune response (9). Emerging data for DAA treatment support a rapid and complete restoration of most innate immune cells in the blood and in the liver with resolution of liver inflammation. However, whether successful DAA treatment will restore HCV-specific immune responses and abolish development of end-stage liver disease is not yet clear. Indeed, recent data suggest a complex picture and several confounding factors that may influence the effect of DAA therapy on immune restoration and spontaneous HCV clearance on reinfection. In this article, we review the current knowledge on this topic.

THE LIVER AS AN IMMUNE ORGAN

The liver is a multifunctional organ exposed to various toxins and nutrients, and it is thus primarily an innate immune organ that plays an early and important role in host defence (10). It is also an immune-privileged organ that favours tolerance (11). The key immunologic function of the liver is immune surveillance, which is dependent on striking a balance between tolerance and activation of both innate and adaptive immunity. The liver is composed primarily of parenchymal cells or hepatocytes (~80%). The remaining 20% are non-parenchymal cells composed of resident innate and adaptive immune cells of lymphoid origin including T cells, natural killer (NK) and natural killer T cells, innate lymphoid cells, and mucosal-associated invariant T cells (MAIT). B cells are also found in the liver and can have a profibrogenic role (12–14). Non-parenchymal cells further include immune cells of myeloid origin, including Kupffer cells (KCs), monocytes, neutrophils, myeloid-derived suppressive cells, and various types of dendritic cells (DCs). Finally, non-parenchymal cells endowed with innate functions such as liver sinusoidal endothelial cells and hepatic stellate cells are also enriched in this organ and are important for immune surveillance, antigen presentation, and tolerance induction.

RESTORATION OF INNATE IMMUNITY AFTER DAA TREATMENT

On infection, liver cells are involved in the sensing and early containment of HCV, induction of inflammatory and antiviral responses, and orchestration of virus-specific adaptive immune responses. Various pattern recognition receptors (PRRs) have the capacity to sense the presence of viral proteins and nucleic acids as pathogen- associated molecular patterns (15,16). PRR-mediated innate signalling (i.e., Toll-like receptors, retinoic acid-inducible gene I (RIG-I)-like receptors) leads to the expression of a set of immediate early protective genes. These include antiviral genes such as type I and III IFNs, chemokines, and pro-inflammatory cytokines, which then trigger IFN-stimulated genes. HCV has developed multiple mechanisms to impair PRR-mediated signalling pathways and to escape innate immune responses. Nevertheless, significant upregulation of IFN-stimulated genes was reported in the livers of HCV-infected patients and chimpanzees (17–19). Chronic HCV infection and persistent IFN-stimulated gene signalling induce activation of innate immune cells in the liver and the peripheral blood, and the resulting virus-driven sustained inflammation is associated with the development of chronic liver disease, including fibrosis, cirrhosis, and HCC.

The fact that HCV is able to establish chronic infection in the presence of such a strong antiviral response suggests that expression of essential genes is disturbed during the early phase of the innate immune response. We conducted studies with paired liver biopsies and corresponding purified hepatocytes isolated from HCV-infected patients undergoing liver transplantation and reported that antiviral response is largely supported by infected hepatocytes (20). Expression of early responsive IFN regulatory factor 3 IRF3-dependent genes (i.e., IFNB1, IL28A/B, CCL5) is severely compromised and is associated with a global decrease in expression of the mitochondrial antiviral-signaling

Table 1: Innate immunity restoration after therapy

Immune compartment and status in chronic HCV	Restoration after successful treatment	
	IFN-based therapy	DAA therapy
Viral sensing (PRR-mediated signalling)		
Impaired signalling Altered early IFN response	Not restored (23)	Restored signalling and response to IFN (21,23)
NK cells		
Low cytokine production ↑ activation ↑ degranulation ↓ response to IFNα	↑ NK function predictive of SVR (24)	Restoration of NK phenotype (25,26)
DC		
↓ Frequency ↓ Pro-inflammatory cytokines ↑ Immunosuppressive cytokines	Impaired DC associated with treatment failure (27)Restored allogenic responses (28)	Restored PRR-mediated defect (29)
Monocytes and macrophages		
↓ IL-18 production Impaired differentiation of monocytes into M1 and M2 macrophages	Not tested	Partial restoration of macrophage polarization (30)
MAIT cells		
↓ Frequency in liverGlobal activation of cells ↓ Response to bacteria	↑ MAIT activation (31) ↑ Frequency (31)	Partial normalization of frequency (32) Reduced liver inflammation (32) No restoration of response to bacteria (32)

Note: ↑ = increased; ↓ = decreased. DAA = Direct-acting antiviral; DC = Dendritic cell; HCV = Hepatitis C virus; IFN = Interferon; IL = Interleukin; MAIT = Mucosal-associated invariant T cell; NK = Natural killer; PRR = Pattern recognition receptor; SVR = Sustained virologic response

adaptor (MAVS) in infected hepatocytes. Moreover, the RIG-I-like receptor pathway was shown to be severely compromised on the restricted expression of the NS3/4A protease, concomitant with mitochondrial antiviral-signaling adaptor proteolytic cleavage in human primary hepatocytes (21). More important, this impairment was completely reversed by treatment with the HCV NS3 protease inhibitor BILN2061 (ciluprevir) (21), the first DAA to be tested in humans (22) (Table 1). Thus, therapeutic clearance of HCV with DAA is expected to restore innate functions of hepatocytes in vivo. In support of this notion, DAA therapy was reported to rapidly reduce inflammation in the livers of mice with humanized livers, a finding that was not associated with IFNα/ribavirin therapy (23).

NK cells are important players in the host response to HCV infection (33). Innate protection from HCV acquisition in exposed individuals was associated with an increased frequency of mature NK cells, higher levels of natural cytotoxicity receptors, and enhanced cytotoxicity (34). Increased NK cell degranulation was also observed in the early phase of HCV infection in people who inject drugs (PWID) who clear HCV spontaneously (35). Spontaneous resolution was associated with increased expression of the activating receptors (NKp44 and NKp46) (36) and decreased expression of the inhibitory receptor NKG2A (35). Moreover, increased NK cell cytotoxicity in the early phase of infection was related to strong T cell responses (35,36). Several studies also report functional impairment of NK cells in chronic HCV infection, where NK cells exhibit an exhausted status with reduced production of effector cytokines (eg, IFNγ) and increased cytotoxicity that can promote liver damage (33).

Although the exact mechanisms leading to NK cell exhaustion are poorly defined, emerging studies show that multiple negative regulatory pathways are involved, such as dysregulated NK cell receptor signalling, as are suppressive effects by regulatory cells or suppressive cytokines (TGF-β) within the microenvironment (reviewed in [37]). Increased NK cell effector function during PEG-IFNα/RBV therapy is predictive of a successful treatment outcome (24). Furthermore, treatment with DAA, asunaprevir (NS3 protease inhibitor), and daclatasvir (NS5A inhibitor) reduced serum levels of the NK cell–stimulating cytokines (interleukin [IL]-12 and IL-18) and the inflammatory chemokines CXCL10 and CXCL11 (25,26). DAA-mediated viral clearance also reversed the activated NK cell phenotype observed in patients with chronic HCV (25,26). This was associated with improved NK cell degranulation on in vitro stimulation with IFNα (38). The improved NK cell responsiveness on rapid reduction of viremia through a DAA regimen, although indicative of functional restoration of NK cells, may also be relevant for improved immune surveillance and prevention of viral relapse.

DCs play a critical role in sensing viruses directly through their PRRs and in linking innate and adaptive immunity (39). As such, chronic viral infections have evolved mechanisms to interfere with pathogen recognition and cytokine production. HCV-induced alterations are detectable in peripheral blood myeloid DCs and plasmacytoid DCs, including reduced frequency, impaired production of inflammatory cytokines, and increased production of the immunosuppressive cytokine IL-10 (40,41). Several investigators independently report impairment of PRR-induced synthesis of cytokines (IL-12, tumour necrosis factor [TNF]-α, IFNα/IFNβ, IL-12p70) from myeloid DCs in chronic hepatitis C (42–44). The HCV-mediated impairment was dependent on cell-associated HCV RNA levels (42) and via select PRRs (TIR-domain-containing adapter-inducing IFNβ [TRIF] and IFNβ promoter stimulator 1 [IPS-1, also called MAVS] pathway) (29). The functional modulation of defective DCs seems to be directly associated with successful response to IFN therapy. Patients who did not respond to PEG-IFNα/RBV therapy had circulating DCs with significantly decreased capacity to stimulate allogeneic T lymphocytes and to produce IL-12 compared with patients who achieved SVR (27). Pertinently, allogeneic responses were decreased in patients with chronic hepatitis C and restored on clearance of cell-associated viral RNA from myeloid DCs after 4 weeks of IFNα/RBV therapy (28). Collectively, these data from IFN-based therapies suggest that rapid and complete restoration of DCs after HCV elimination is possible. More important, the PRR-mediated defect induced by HCV could be restored in vitro by treatment with DAA coincident with reduction in cell-associated viral load (29). Further studies are required to establish the functional restoration of DC after DAA therapy in vivo.

Monocytes and macrophages are essential players in the inflammatory response. Monocytes sense HCV-infected cells and produce IL-18 via the inflammasome. IL-18 is essential for NK and MAIT cell activation (45). Patients with chronic HCV exhibit reduced monocyte function, leading to impaired NK cell IFNγ-mediated responses (45). Furthermore, HCV core protein suppressed in vitro differentiation of monocytes into either M1 or M2 macrophages through Toll-like receptor 2 activation (45). An interesting finding is that DAA-mediated HCV clearance (NS5A inhibitor) partially restored the impaired macrophage polarization (30). KCs are liver-resident macrophages particularly relevant in chronic HCV infection. However, access to liver samples is challenging, and therefore most studies are conducted with either KCs isolated from healthy liver (obtained from a transplantation setting) or monocyte-derived macrophages that are then exposed to HCV antigen or Toll-like receptor ligands in vitro. Studies with KCs isolated from healthy liver suggest a role in inducing immune tolerance to HCV, including the production of IL-10 (46,47). However, evidence suggests that KCs help control HCV infection through cytokine production (IL-1β, TNF-α), synergistic effects with other cells, and mediation of virus-specific immunity (46,48). TNF-α obtained from either KCs isolated from healthy liver or monocyte-derived macrophages was reported to promote viral infectivity (HCVcc and HCVpp) in vitro (49). Nevertheless, little evidence has been reported to elucidate evasion mechanisms that might induce chronicity of HCV infection and whether treatment with IFN or DAA could restore aberrantly activated KCs' function on HCV eradication (50).

MAIT cells are innate-like lymphocytes that are activated during HCV infection in a T cell receptor–independent pathway but reliant on IL-18 in synergy with IL-12, IL-15, or IFNα or IFNβ,

resulting in cytokine release and granzyme B up-regulation (31). In chronic HCV infection, MAIT cells are activated by monocyte-derived cytokines and impaired in their response to bacteria. MAIT cells exhibit reduced frequencies, activated phenotype, altered transcription factor expression, and reduced response to their cognate MR1-dependent antigen stimulation in the blood of patients with HCV (51). These defects were not reversed after HCV elimination by DAA therapy (51). The frequency of MAIT cells in the liver was also significantly decreased and correlated inversely with liver inflammation and fibrosis (32). DAA therapy resulted in a rapid decrease in liver inflammation and MAIT cell activation and an increase in intrahepatic MAIT cell frequency that remained below the frequencies in uninfected controls (32). It is interesting that IFNα-based therapies have recently been shown to increase MAIT cell activation and frequency in treated HCV patients, most likely reflecting a direct type I IFN-mediated effect on MAIT cells (31).

In summary, data to date suggest efficient restoration of innate immune functions in DAA-cured individuals with the exception of MAIT cells. Future studies are required that dissect the molecular mechanisms (eg, cytokine normalization vs antigen removal) underlying the differential responsiveness of these immune cell subsets and others to DAA-mediated clearance of HCV.

RESTORATION OF ADAPTIVE IMMUNITY AFTER DAA TREATMENT

Spontaneous resolution of acute HCV infection is characterized by the early emergence of HCV-specific CD8 T cells expressing the IL-7 receptor alpha (CD127), a marker of memory T cells, and the anti-apoptotic molecule Bcl-2 (reviewed in [1,52]). This response is typically of high magnitude, broad (targeting multiple HCV epitopes), and polyfunctional (producing multiple cytokines and displaying proliferative and cytotoxic properties). In contrast, infections that progress to chronicity are characterized by a CD8 T cell response of low-frequency, narrow, or impaired effector functions (1). This limited immune response may transiently control viremia, but the immune selection pressure it exerts leads to the emergence of viral escape mutants within targeted epitopes, thus facilitating viral persistence. CD8 T cells targeting epitopes that have mutated revert to a memory-like phenotype ($CD127^{hi}$) because they no longer recognize the autologous virus. Chronic antigen stimulation of CD8 T cells recognizing intact epitopes induces T cell exhaustion and expression of a spectrum of inhibitory receptors, including programmed death-1 (PD1), T cell immunoglobulin and mucin-domain-containing-3 (TIM-3), cytotoxic T-lymphocyte protein 4 (CTLA-4), 2B4, CD160, killer cell lectin-like subfamily G member 1 (KLRG1), T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT), and CD39 (53). This is coupled with increased expression of the transcription factor eomesodermin (Eomes) and down-regulation of the T-box transcription factors (T-bet) and cell surface expression of CD127 (54). Expression of the transcription factor T cell factor 1 (TCF1) distinguishes a subset of exhausted virus-specific CD8 T cells with memory-like properties (55). T cell exhaustion results in progressive loss of effector functions with limited cytokine production, proliferation capacity, and eventually disappearance of the majority of HCV-specific CD8 T cells from peripheral blood and their localization to the liver (56,57). Exhaustion of bystander CD8 T cells targeting other viruses has been observed in individuals with chronic HCV infection, especially in the liver (58).

PEG-IFNα/RBV treatment during the first 6 months after HCV infection was associated with reconstitution of $CD127^{hi}$ $Bcl2^{hi}$ polyfunctional HCV-specific memory CD8 T cells, but late treatment, after years of chronic infection, failed to reconstitute a similar response (9,59–64) (Table 2). IFN-free DAA treatment with a combination of faldaprevir (NS3 protease inhibitor) and deleobuvir (non-nucleoside NS5B polymerase inhibitor) with or without ribavirin led to a rapid restoration of the in vitro proliferative capacity of HCV-specific CD8 T cells and a slight reduction in the ex vivo frequency of $PD1^+$ HCV-specific CD8 T cells (65). Similarly, a regimen of daclatasvir, asunaprevir, and beclabuvir (NS5B polymerase inhibitor) demonstrated a partial reduction in the expression of PD1 on HCV-specific CD8 T cells at 24 weeks after the end of treatment (66).

Recently, $TCF1^+CD127^+PD1^+$ HCV-specific CD8 T cells expressing both exhaustion and memory markers were described in subjects with chronic infection. This T cell subset was maintained in subjects treated with different DAA regimens during and after treatment (67). Interestingly, this population expanded in one subject

Table 2: Adaptive immunity restoration after therapy

Immune compartment and status in chronic HCV	Restoration after successful treatment	
	IFN-based therapy	DAA therapy
CD8		
↓ Proliferation ↓ Cytokine production (IFNγ, TNF-α, IL-2) Exhaustion profile (PD1, TIM-3, CTLA-4, 2B4, CD160, KLRG1, TIGIT, CD39)	Restored only if treated early (9)	↑ Proliferation (65) ↓ PD1 expression (65,66) No restoration of CD127+PD1− HCV-specific CD8 T cells (67)
CD4		
↓ Proliferation ↓ Cytokine production (IFNγ, TNF-α, IL-2) Exhaustion profile (PD1, TIM-3, CTLA-4)	↑ IFNγ responses (68) Transient or no restoration (69,70) Require further investigation	Bulk population (66): ↓ TIGIT ↑ Memory phenotype ↑ T-bet/Eomes ratio HCV-specific: require further investigation
Treg		
↑ Frequency ↑ Hepatic infiltration	Not normalized (71,72)	Not normalized (73)
B cells		
B cell abnormalities (cryoglobulinemia vasculitis) ↑ Frequency of IgM+CD21− B cells		↓ Symptoms (74,75) Normalization of B cell phenotype (74,75) (↓ % of IgM+CD21− B cells) (74,75)

Note: ↑ = increased; ↓ = decreased: CTLA-4 = Cytotoxic T-lymphocyte protein 4; DAA = Direct-acting antiviral; Eomes = eomesodermin; HCV = Hepatitis C virus; IFN = Interferon; IL = Interleukin; PD1 or CD279 = Programmed death 1; T-bet = T-box transcription factors; TIGIT = T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains; TIM-3 = T cell immunoglobulin- and mucin-domain-containing-3; TNF = Tumour-necrosing factor; Treg = Regulatory CD4 T cells

who relapsed and differentiated into terminally exhausted TCF1^{-}CD127^{-}PD1^{hi} HCV-specific CD8 T cells. However, these memory-like CD8 T cells were distinct from conventional memory T cells detected in spontaneously resolved subjects because they expressed higher levels of Eomes and TCF1. They also produced lower levels of IFNγ and TNF-α on in vitro stimulation of peptide-expanded HCV epitope-specific CD8 T cells. Finally, the CD127^{+}PD1^{-} HCV-specific CD8 T cell subset, abundant in spontaneously resolved individuals, did not recover in DAA-treated subjects (67). Together, these studies suggest partial reversal of HCV-specific CD8 T cell exhaustion state after DAA treatment. Whether early treatment during acute infection would reconstitute a better immune response as observed in IFN-based therapy remains to be investigated. It is also essential to compare immune reconstitution and reversal of exhaustion in the liver versus peripheral blood, but it will be challenging because of the limited capacity to isolate circulating HCV-specific CD8 T cells, notably in individuals with advanced liver fibrosis (76).

CD4 helper T cells are essential for maintaining the proliferation and polyfunctionality of HCV-specific CD8 T cells and successfully clearing the infection (reviewed in [1,52]). A vigorous, broad, and sustained virus-specific CD4 T cell response is associated with spontaneous clearance of HCV infection. Although the majority of individuals with acute HCV infection do generate a virus-specific CD4 T cell response, it is typically of limited breadth. Chronic infection is associated with rapid exhaustion of HCV-specific CD4 T cells characterized by an increased expression of PD1, CTLA-4, and TIM-3 and gradual loss of the proliferative capacity and reduced production of IL-2, TNFα, IFNγ, and IL-21. Follicular helper T (Tfh) cells, crucial for germinal centre formation and development of high-affinity antibodies, were detected in

the blood of patients with acute infection but were also highly enriched in the liver during chronic HCV infection (77,78). The exact contribution of Tfh cells in mediating spontaneous HCV clearance is not yet clear.

It has been difficult to examine immune restoration of HCV-specific CD4 T cells because they are functionally impaired in individuals with chronic infection. A restricted set of major histocompatibility complex (MHC) class II tetramers of the most common helper epitopes is available, but the low frequency of virus-specific CD4 T cells has limited their use. Restoration of HCV-specific CD4 function during IFN-based therapy is controversial, with some studies demonstrating an enhanced response in IFNγ Enzyme-Linked ImmunoSpot (ELISPOT) assays in patients who achieve SVR (68), and others showing a transient restoration or no effect altogether (69,70).

DAA treatment was associated with reduced expression of TIGIT—a shift from central ($CD45RA^{-}CCR7^{+}$) to effector ($CD45RA^{-}/CCR7^{-}$) memory T cell phenotype and an increased T-bet/Eomes ratio—in bulk CD4 T cells, characteristic of effector functions (66). However, restoration of HCV-specific CD4 T cell numbers or functionality was not examined, and it would be imprudent to make solid conclusions solely on the basis of these data. Similarly, immune restoration of both CD4 and CD8 T cells in the liver remains an open question. This may be difficult to address because of the shift to a noninvasive grading of liver fibrosis and ethical constraints in obtaining liver biopsies from patients treated with DAA.

Regulatory CD4 T cells (Treg cells; $CD25^{+}FoxP3^{+}$CD4 T cells) regulate both innate and adaptive immunity and are expanded in the peripheral blood and liver of individuals with chronic HCV infection (79,80). Increased intrahepatic infiltration of Treg cells and their activation may limit fibrosis through the production of IL-10 (80,81). However, Treg cells may also correlate with advancing fibrosis by limiting the antifibrotic activity of NK cells. Most studies of IFN-based treatment suggest that SVR does not significantly alter the intrahepatic infiltration or activation status of Treg cells for up to 4 years after the end of treatment (71,72). To date, only one study has examined Treg cells in the peripheral blood of patients treated with DAA either in combination with IFN or not (73). In both regimens, the frequency of Treg cells did not normalize up to one year after the end of treatment (73). Longer follow-up is needed to fully understand the persistence of these immunosuppressive Treg cells in the liver and their contribution to liver disease.

B cells are not well characterized in HCV infection. On one hand, increasing evidence suggests that anti-HCV neutralizing antibodies are associated with spontaneous clearance of primary HCV infection and reinfection (82–84). Yet, on the other hand, neutralizing antibodies were detected several weeks after infection regardless of the infection outcome, suggesting that the humoral immune response is not the sole contributor to viral clearance (reviewed in [1]). Chronic HCV infection is associated with several B cell–related abnormalities. Circulating mixed cryoglobulins are detected in 40%–60% of patients, and overt cryoglobulinemia vasculitis (CV) occurs in 5%–10% of cases (74). IFN-free DAA treatment was shown to be effective and associated with reduced symptoms in patients with CV. The kinetics of B cells were examined in a prospective study of patients with chronic HCV–CV treated with sofosbuvir (NS5B polymerase inhibitor) plus ribavirin, sofosbuvir plus daclatasvir, or sofosbuvir plus simeprevir (NS3 protease inhibitor) (74) and in a multicenter study of sofosbuvir plus daclatasvir (75). HCV–CV patients had a reduced frequency of $CD4^{+}CD25^{hi}FoxP3^{+}$ Treg cells compared with healthy donors, but higher frequencies of $IgM^{+}CD21^{-}$ and low-memory B cells, Th17 CD4 T cells, and $CD4^{+}CXCR5^{+}IL\text{-}21^{+}$ Tfh cells before treatment. DAA therapy increased the frequency of Treg cells and reverted the expansion of $IgM^{+}CD21^{-}$ and low memory B cells, Th17, and Tfh cells (74,75). Additional studies with other cohorts and more detailed immunological characterization are needed to understand the impact of DAA treatment on different B cell subsets, B cell lymphoproliferative disorders in HCV, and neutralizing antibodies.

DAA-INDUCED PROTECTION FROM CHRONIC INFECTION AFTER HCV REEXPOSURE

The risk of HCV reinfection after spontaneous or treatment-induced viral clearance remains a problem among individuals with high-risk behaviours, such as PWID (85,86). Spontaneous resolution of acute infection leads to the generation of HCV-specific long-lived memory T cells that can protect against viral persistence on reexposure. Although

sterilizing immunity may be rare, humans and chimpanzees that are reexposed to HCV exhibit reinfection episodes of shorter duration and reduced viremia compared with primary infection (82,87,88). Protective immunity on reinfection was characterized by expansion of broad (targeting multiple epitopes) and polyfunctional memory CD4 and CD8 T cells (89,90) and generation of cross-reactive antibodies (82). In contrast, failure to recognize the reinfection viral variants and expression of variable levels of the exhaustion marker PD1 was associated with failure to clear HCV reinfection and viral persistence (89).

DAA treatment does not protect against HCV reinfection, which is estimated at a rate of 4.6 per 100 person-years in a trial of elbasvir (NS5A inhibitor) and grazoprevir (NS3 protease inhibitor) therapy for 12 weeks (91,92). As discussed earlier, it is not clear whether treatment-induced HCV clearance is associated with the generation of comparable HCV-specific memory immune responses that may enhance the chance of clearing a subsequent HCV infection. A limited number of spontaneous resolution cases after a second homologous or heterologous reinfection were reported in IFN-treated PWID (93). Because early, but not late, IFN treatment was associated with better restoration of HCV-specific memory T cells, it is tempting to speculate that a similar phenomenon is likely with DAA. Such a well-restored immune response may provide better protective immunity and higher rates of spontaneous clearance on reinfection. Studies comparing immune reconstitution in PWID who received DAA in acute versus chronic HCV infection and after treatment monitoring of reinfection are required to validate this hypothesis.

POTENTIAL OF HCV VACCINATION IN DAA-TREATED INDIVIDUALS

Vaccine development against HCV remains a research priority (85,94). One vaccine targeting HCV-specific T cells is currently in clinical trials in PWID (NCT01436357) (95), and another targeting humoral responses (96) is set to start in the near future. These two vaccines have demonstrated very good efficacy in healthy donors. A key question is whether such vaccines will provide protective immunity in DAA-treated individuals. The immune system of these individuals has already failed once, and whether such failed immune response can be resuscitated is not clear. Vaccination with a recombinant chimpanzee adenoviral vector (ChAd3) and a modified vaccinia Ankara (MVA) encoding the nonstructural polyproteins of HCV (NSmut) prime/boost, a vaccine regimen that has demonstrated impressive immunogenicity in healthy volunteers, could not reconstitute HCV-specific T cell immunity in patients with chronic HCV infection (97). Nevertheless, de novo T cell responses were induced when there was a sequence mismatch between the autologous virus and the vaccine immunogen. This suggests that it may be possible to prime similar de novo immune responses using novel vaccine antigens and appropriate formulations.

DAA-INDUCED IMMUNE RECONSTITUTION AND EFFECT ON HCC

The risk of developing HCV-related HCC increases with advanced cirrhosis. Data from IFN-based therapy indicate a reduced risk of developing HCC among patients who achieved SVR versus those who did not. Thus, it was assumed that DAA would have a similar impact. However, data emerging from early DAA trials have been conflicting, with some demonstrating higher risk of HCC recurrence and development despite curative DAA therapy. Several confounding factors are implicated that may explain the discordance between these studies; they are discussed elsewhere (98,99). More recent data from a larger cohort (22,500 patients) suggest that achieving SVR was associated with a lower risk of HCC, but the risk remains highest in individuals with advanced cirrhosis or prior HCC (100). Hence, there is a need to develop prognostic markers that may predict the risk of developing HCC before and after DAA treatment. A set of liver gene signatures was identified and shown to predict post-SVR development of HCC (99). Similarly, a small study of 13 patients, 3 of whom developed HCC, identified a panel of nine cytokines, measured in serum before treatment (monokine induced by γ IFN [MIG], IL-22, TNF-related apoptosis-inducing ligand [TRAIL], A proliferation-inducing ligand [APRIL], vascular endothelial growth factor [VEGF], IL-3, tumour necrosis factor-like weak inducer of apoptosis [TWEAK], stem cell factor [SCF], IL-21), that distinguished patients who developed de novo HCC (101). Although promising, this interesting finding remains to be validated with larger cohorts. Recent RNA-seq and ChIP-seq analyses have demonstrated that

HCV infection induces marked changes in histone modifications on a genome-wide level in cell culture and in livers from HCV-infected individuals. Curative DAA therapy resulted in only a partial reversal of virus-induced transcriptional changes. Furthermore, cured cells exhibited the previously described liver gene signatures associated with a risk for developing HCC (102). Altogether, these data suggest that HCV infection may induce irreversible changes in the liver microenvironment that may continue to enhance the risk of developing HCC.

For immune reconstitution after curative DAA therapy, it will be important to distinguish between individuals who have no previous history of HCC and those who do. Among the former, it is likely that viral elimination will reduce intrahepatic inflammation that promotes oncogenic transformation. Furthermore, DAA treatment will reconstitute innate immune mediators such as NK cells and slightly MAIT cells (see above) that have important immune surveillance characteristics against cancers. In the case of individuals with a history of HCC, the effect on T cells targeting tumour-associated antigens (TAAs) or neoantigens will likely be important. As discussed earlier, limited data suggest reduced activation of bulk lymphocytes after DAA therapy (66), but the effect on TAA-specific T cells is unknown. With the recent approval of nivolumab, a PD1 inhibitor, for treatment of HCC (103), it is crucial to understand how DAA treatment reshapes the immune landscape in the liver, the exhaustion status of TAA-specific tumour-infiltrating lymphocytes, and the expression of inhibitory ligands (eg, PDL1) on liver cells. Data from hepatitis B–related HCC has suggested that HCC TAA-specific T cells are more exhausted in these individuals than are HCC TAA-specific T cells from other etiologies (104), whereas a more recent study demonstrates no difference (105).

Finally, preliminary studies suggest that Treg cells do not normalize in the peripheral blood after DAA treatment. Additional analysis with larger cohorts and in the liver is warranted. The frequency of blood and tumour-infiltrating Treg cells correlates with CD8 T cell dysfunction and poor survival in HCC patients (106). If DAA therapy does not significantly alter the localization and suppressive activity of intrahepatic Treg cells, this will create an immune-suppressive environment permissive to tumour growth.

FUTURE DIRECTIONS

The introduction of DAAs has caused an enormous paradigm shift in the treatment of chronic HCV infection. DAAs have a huge potential to prevent progressive liver disease. Despite the high cure rates, continued clinical follow-up, and studies of the immune response, liver disease progression and prophylactic HCV vaccination are still required for high-risk and marginalized populations. Priorities include mechanistic studies on immune restoration in individuals treated during acute or chronic HCV and understanding the molecular mechanisms associated with development of HCC and how these mechanisms are affected by DAA. Studies of the evaluation of the extent of immune restoration according to liver fibrosis stage are also very important to better understand the effect of fibrosis on immune dysfunction as well as to strengthen the argument that early treatment has increased benefits. Cohort studies are required to monitor reinfection in DAA-treated high-risk individuals. In addition, vaccination of DAA-cured individuals and their inclusion in clinical trials is essential to validate the efficacy of vaccines in long-term protection from re-infection after DAA cure. Finally, long-term systematic follow-up of all DAA-treated patients through large cohort studies, administrative databases, and registries is essential. The data obtained will provide better estimates of the rates of fibrosis regression, incidence of HCC, and impact of treatment on limiting the burden of HCV-related liver disease in the long term.

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