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REVIEW

Programmed death-1/programmed death-L1 signaling pathway and its blockade in hepatitis C virus immunotherapy

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Abstract

Chronic hepatitis C virus (HCV) infection is a public health issue that often progresses to life-threatening complications, including liver cirrhosis, fibrosis, and hepatocellular carcinoma. Impaired immune responses to HCV are key features of chronic HCV infection. Therefore, intervention strategies usually involve enhancing the immune responses against HCV. Cytotoxic $CD8^+$ T lymphocytes (CTLs) play a critical role in the control of HCV infection. However, their cytolytic function can be impaired by the expression of co-inhibitory molecules. Programmed death-1 (PD-1) receptor and its ligand PD-L1 function in a T cell co-inhibitory pathway, which either blocks the function of CTLs or the differentiation of $CD8^+$ T cells. During chronic HCV infection, the immune inhibitory receptor PD-1 is upregulated on dysfunctional HCV-specific $CD8^+$ T cells. As such, blockade of the PD-1/PD-L1 pathway in these $CD8^+$ T cells might restore their functional capabilities. Indeed, clinical trials using therapies to block this pathway have shown promise in the fostering of anti-HCV immunity. Understanding how chronic HCV infection induces upregulation of PD-1 on HCV specific T cells and how the PD-1/PD-L1 interaction develops HCV specific T cell dysfunction will accelerate the development of an efficacious prophylactic and therapeutic vaccination against chronic HCV infections, which will significantly improve HCV treatments and patient survival. In this review, we discuss the relationship between PD-1 expression and clinical responses and the potential use of PD-1 blockade for anti-HCV therapy.

Key words: Hepatitis C virus; Programmed death-1; Hepatitis C virus immunotherapy; Exhausted T cells; Hepatitis C virus immune escape

Core tip: The programmed death-1 (PD-1)/PD-L1

pathway is an attractive target for anti-hepatitis C virus (HCV) immunotherapy because it restores the functional capacities of HCV-specific T cells. This is an extremely promising development in anti-HCV vaccines research since restoration of exhausted anti-HCV T cells is a major challenge when developing either prophylactic or therapeutic vaccines. This review will discuss the correlation between PD-1 expression and the clinical outcome in HCV patients and how this information can be potentially applied to block PD-1/PD-L1 pathway for HCV immunotherapy.

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INTRODUCTION

Chronic viral infections, including hepatitis C virus (HCV), hepatitis B virus (HBV), and human immunodeficiency virus (HIV), are among the main causes of death worldwide[1]. While most viral infections prompt successful T cell responses that remove the infections, HCV, HBV, and HIV have acquired mechanisms to avoid immune elimination, permitting them to persist in many, if not all, infected individuals. These escape mechanisms lower the responsiveness of patients to anti-viral therapy.

HCV is found in nearly every region of the world, affecting an estimated 170 million patients and 1%-2% of the overall population in most infected countries[2]. HCV not only causes hepatitis C, but it also provides the perfect infection setting to study viral evasion mechanisms, since the infection persists in most infected individuals while 25% of infected patients effectively clear the virus. This allows for the comparison of immune responses between responders and non-responders. How these immune responses determine whether a patient eliminates infection or develops a chronic infection is not completely understood. Accordingly, viral escape from immune cells has been suggested as a contributing factor to HCV as well as HIV and HBV infection.

In the acute stage of HCV infection, 20%-40% of patients improve spontaneously[3], and this recovery is associated with a robust, HCV-specific T cell responses[4-6]. The discriminating role of the HCV specific $CD8^+$ cells responses in the unprompted recovery of acute HCV infection was demonstrated in chimpanzees[5,6], the only animal model for the study of HCV. Even in chimpanzees with chronically developing, acute HCV infection, intrahepatic infusion of $CD8^+$ T cells promoted a partial decline in the HCV load[7,8]. In chimpanzees, vaccination with an experimental prophylactic vaccine induced HCV-specific $CD8^+$ cell responses and suppression of acute HCV infection[9]. These studies led to the hypothesis that recovery from HCV may be due to induction of HCV-specific T cell responses. Hence, research efforts for the development of novel treatments for chronic HCV infection have focused on T cell responses.

Dysfunction of virus-specific $CD8^+$ cells is a fundamental property of persistent viral infections like HCV; consequently, restoration of T cell capacity is a major aim in the generation of immune-based therapies for persistent infection of viruses[10]. Many factors are known to contribute to T cell dysfunction, including inhibitory cytokines, regulatory T cells, and inhibitory receptors expressed on T cells[10]. Accordingly, removal or blockade of these inhibitory factors may be a promising approach for the treatment of persistent viral infection.

Overall, the mechanisms that have been proposed to date to explain impaired immunity in chronic HCV infection are summarized as follows: (1) HCV escapes immune responses by developing mutations; (2) primary T cell exhaustion after an extensive response; (3) impaired antigen presentation of dendritic cells (DCs); (4) impaired natural killer (NK) cell activities; (5) skewing the Th1 type cytokine to a Th2 type; (6) suppression by HCV proteins; (7) impaired T cell maturation; (8) suppression by regulatory T cells; (9) the nature of the tolerogenic environment in the liver; and (10) the expression of co-inhibitory molecules on immune cells[11].

An important inhibitory receptor that downregulates T cell function is programmed death-1 (PD-1)[12]. PD-1, with its two known ligands B7-H1/PD-L1 and B7-DC/PD-L2, has recently been shown to be upregulated on HCV- and HIV-specific $CD8^+$ cells, indicating that PD-1 upregulation may be an essential mechanism for viral immune escape in chronic HCV and HIV infections[13-17].

Here, we provide up to date review on the role of PD-1 in HCV immune evasion and the potential use of PD-1 blockade for anti-HCV therapy. Understanding the relationship between PD-1/PD-L1 and T cell dysfunction and its role in HCV persistence will accelerate the development of an efficacious prophylactic and therapeutic vaccination against chronic HCV infection.

HCV TREATMENT AND FAILURE

Combination therapy with pegylated interferon (PEG-IFN) and ribavirin (RBV) is the current standard therapy for individuals with chronic HCV infection[18-21]. Treatment duration is 48 wk for HCV genotypes 1 and 4, and 24 wk for genotypes 2 and 3. The dominant majority of treated patients, especially those with HCV genotypes 2 or 3, show a significant virologic response. Almost 66% of patients with HCV genotype 2 or 3 accomplish rapid virologic response (RVR), characterized by untraceable HCV RNA within 4 wk of starting treatment, and 97% have undetectable HCV RNA within 12-24 wk of starting treatment. Seventy-six percent attain sustained virologic response (SVR)[21,22]. Unfortunately, only approximately half of all patients accomplish SVR with 24-48 wk of therapy with PEG-IFN and RBV[18,20].

Factors that contribute to non-responsiveness, other than genotype, are high baseline HCV viral-load, high fibrosis stage in the liver, male gender, old age, race, obesity, alcohol intake, insulin resistance, liver steatosis, and alterations in the host immune response, such as high interleukin (IL)-8 and IL-10 serum levels[23-25].

Current IFN-based therapy does not work in many patients, possibly due to a combination of viral and host factors. Innate immunity to HCV is activated by cellular sensors that identify the presence of pathogen-associated molecular patterns (PAMPs). Key fundamental cellular sensors for HCV infection are toll-like receptor 3 (TLR3), which recognizes double-stranded RNA (dsRNA), and the RNA helicase retinoic acid-inducible gene 1 (*RIG-1*). The HCV PAMP sensors TLR3 and RIG-1 signal through the adaptor proteins TIR-domain-containing adapter-inducing interferon-β (TRIF) and Cardif, respectively. Remarkably, the HCV NS3-4A serine protease relieves both Cardif[26,27] and TRIF[28] to disable signals initiated by RIG-1 and TLR3. In addition to blockade of the upstream events of IFN-β transcription by NS3-4A, there is evidence that other HCV proteins block IFN signaling downstream of the IFN-α/β receptor. Overexpression of HCV core protein causes activation of suppressors of cytokine signaling (SOCS) 3 protein[29], which in turn hinders signal transducer and activator of transcription 1 (STAT1) phosphorylation by janus kinase 1 (Jak1). These mechanisms support viral persistence even in the face of IFN-based therapies. Further understanding of the molecular mechanisms underlying HCV resistance to the host immune response will lead to generation of novel therapeutic strategies. Moreover, host factors, such as insulin resistance and race, have considerable effects on treatment responsiveness. Adjustment of adverse host factors, whenever possible, may be a feasible alternative for the optimization of HCV therapy.

PEG-IFN is contraindicated in decompensated cirrhosis[30] and is associated with constitutional, autoimmune, neuropsychiatric, and hematological side effects[31], whereas RBV is contraindicated in renal failure[32] and is associated with rash, cough, hemolysis, and teratogenesis[31]. Therefore, many patients are ineligible for or intolerant to PEG-IFN and RBV therapy. However, the approval of sofosbuvir (Sovaldi®, Gilead Sciences), which is a direct acting pyrimidine nucleotide analog that represents the first NS5B HCV polymerase inhibitor, is considered a key step towards a new era in chronic hepatitis C therapy. It was among the first approved antiviral agents with strong activity and high genetic barrier against all HCV genotypes. Additionally, its safety profile is highly favorable, even when it is prescribed to patients with very advanced liver disease and high risk of complications (*e.g.*, cirrhosis with portal hypertension and liver transplant recipients).

ANTI-HCV IMMUNITY

Impaired immune responses to HCV are hallmarks of chronic HCV infection. Therefore, intervention approaches commonly include those that can boost the immune responses against HCV. These immunotherapies for chronic HCV infections include anti-HCV neutralizing antibodies, antagonists of T cell inhibitory factors, therapeutic vaccines, agonists for TLRs, and cytokines[33]. These therapies can be utilized alone or in combination with other antiviral drugs for chronic HCV therapy.

To date, immune-based therapies have not demonstrated satisfactory efficacy. In general, a virologic response was shown only in a small group of patients, and in these cases, the effect was marginal and transient. A critical reason for the poor efficacy is the inadequate activation and stimulation of immune responses. It should be mentioned, however, that the virologic responders showed the strongest T cell responses in a late study that tested the peptide vaccine IC41[34]. This observation demonstrated that a sufficient virologic response might be accomplished by sufficient activation and stimulation of the immune system. Thus, enhancement of the protocol/regimen is needed to improve the efficacy of immune-based therapies.

One possible critical mechanism underlying the inability of HCV patients to resolve the infection is the imbalance between the stimulatory and regulatory immune cells[35]. The poor adequacy of immune-based therapies may be due to various factors. First, many individuals with chronic HCV infection have delayed impairment of the anti-HCV immune response, and it seems unlikely that longstanding immune dysfunction can be repaired by immune-based therapies. Second, HCV advances quickly, and persistent HCV infection brings about the specific survival of viruses that are most proficient at evading host immune responses. Accordingly, these viruses may have the capacity to resist clearance despite the improvement of immune responses by immunotherapies. Finally, the poor adequacy may be credited, in part, to the selection of patients.

Combination therapy might be an efficient strategy for improving the efficiency of immunotherapies For instance, the impact of therapeutic vaccines could be enhanced by combining them with antagonists of T cell inhibitory factors and/or agonists of TLRs. Interestingly, combining antagonists of IL-10R or PD-1 with a therapeutic vaccine strengthened the effects in a murine model of persistent lymphocytic choriomeningitis virus (LCMV) infection[36]. In general, immunotherapies have been well-endured and have not been associated with severe adverse effects. However, improvements in immunotherapies aiming to prompt stronger immune responses may aggravate liver injury and cause severe hepatitis in extreme situations. In this regard, it would be useful to demonstrate the differences between cytotoxic virus-clearing and tissue-damaging T cell responses. A better understanding of the cellular and molecular mechanisms implicated in T cell dysfunction will pave the way for highly efficacious immunotherapies for chronic HCV.

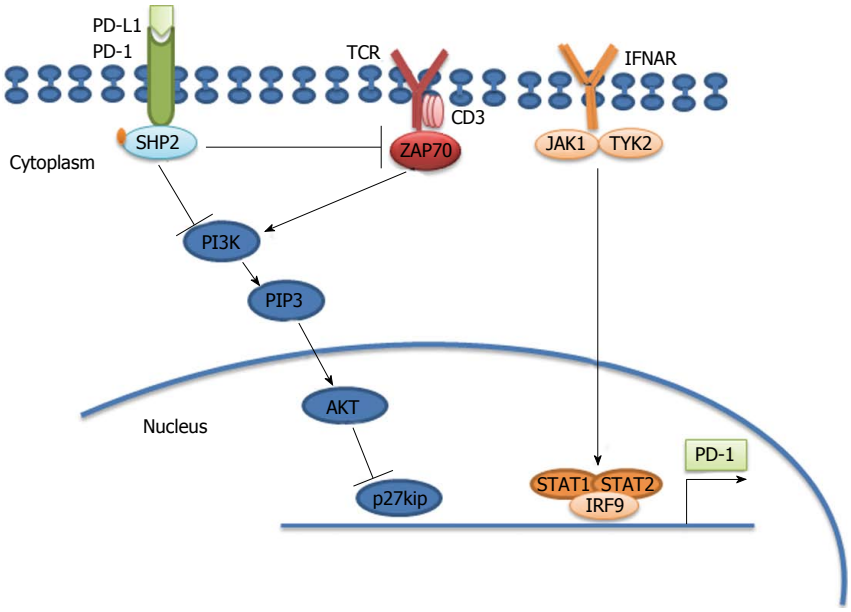

Figure 1 Programmed death-1 causes T cell exhaustion. Programmed death-1 (PD-1) inhibits the T cell receptor (TCR) signaling pathway through src homology 2-containing protein-tyrosine phosphatase 2 (SHP2). PD-1 is located in the immune synapse at the T cell-antigen presenting cell (APC) interface. When its physiological ligand (PD-L1 or PD-L2) binds, PD-1 suppresses the activation and function of T cells through the recruitment of SHP-2, which dephosphorylates and inactivates ZAP70, a major integrator of TCR-mediated signaling. In chronically activated ("exhausted") T cells, interferon (IFN)-α causes overexpression of PD-1 through the binding of the transcription factor IRF9 to the signal transducer and activator of transcription (STAT)1 and STAT2 promoters. PD-1 also results in accumulation of p27kip1, which is an inhibitor of cyclin dependent kinases to block cell cycle and proliferation[84]. ZAP70: Zeta-chain (TCR) associated protein kinase 70 kDa; IRF9: Interferon regulatory factor 9; JAK1: Janus kinase 1.

PD-1 EXPRESSION ON IMMUNE CELLS IN HCV PATIENTS

PD-1 and its ligands play a critical role in the inhibition of the immune system by banning the activation of T-cells, which subsequently decreases autoimmunity and advances self-tolerance. The inhibitory effect of PD-1 is achieved through a dual mechanism of inducing apoptosis in antigen specific T-cells in lymph nodes and decreasing apoptosis regulatory T cells (Tregs) (Figure 1). New classes of drugs that block PD-1, such as Nivolumab, Pembrolizumab, Pidilizumab, and BMS-936559, activate the immune system to attack cancers and are used to treat tumors.

PD-1 has two ligands-PD-L1 (B7-H1)[37,38], which is largely expressed on both hematopoietic and parenchymal cells, and PD-L2 (B7-DC)[39,40], which is mainly expressed on macrophages and DCs. Barber *et al*[12] found that PD-L1 was expressed at very high levels in splenocytes from persistently infected mice, particularly on virally infected cells. Consequently, not only did the exhausted cytotoxic T cells express high levels of PD-1, but its ligand was upregulated on infected cells (Figure 1). PD ligands are differentially regulated, where IFN-γ primarily stimulates PD-L1 expression and IL-4 stimulates PD-L2 expression[41,42]. Recent studies showed that antibody-mediated interference with PD-1 caused regression of several tumor types, including melanoma, renal-cell cancer, and non-small-cell lung cancer, in some patients[43,44]. The inhibitory effect of PD-1 is achieved through a dual mechanism that involves simultaneous induction of apoptosis in antigen specific T-cells in lymph nodes and decreasing apoptosis in regulatory T cells[45,46] (Figure 1).

In the acute stage of HCV infection, HCV specific T cells have been shown to be inadequately functional regardless of the final outcome of the disease[47-51]. A possible mechanism directing this behavior of the HCV-specific T response is exhaustion. When T cells are chronically exposed to high antigen loads, the PD-1/PD-L1 ligand pathway may play a role in T-cell exhaustion. Blocking the PD-1/PD-L1 interaction can permit restoration of exhausted T cells[12,52-55]. These studies indicated that high expression of the inhibitory PD-1 receptor appears to be a signature of functional T cell exhaustion.

Kasprowicz *et al*[17] have demonstrated elevated PD-1 expression on almost all HCV specific $CD8^+$ and $CD4^+$ T cells through the early phase of acute infection, irrespective of clinical outcome or viral load. They also showed that PD-1 expression is reliant on the tissue microenvironment, where the T cells execute their antiviral functions. Interestingly, the overall PD-1 expression levels of infiltrating $CD8^+$ and $CD4^+$ T lymphocytes in the liver were significantly higher compared to peripheral blood[17]. The mean PD-1 expression level on most of $CD8^+$ T liver-residing lymphocytes was 71%, while the median expression on peripheral blood $CD8^+$ T cells from the same subjects was 33%. For liver-derived $CD4^+$ T lymphocytes, the median PD-1 expression level was 53%, while PD-1 expression for cells in the peripheral blood was only 25%[17].

It has been shown that chronic HCV infection has a wide effect on PD-1 expression. For example, PD-1 is

Table 1 Differences between the studies made on the correlation between programmed death-1 expression and clinical outcome

Ref.	No. of patients	Mode of transfection	Symptoms	PD-1 expression levels in acute infection	PD-1 expression levels in resolved patients
Urbani *et al*[16]	19	Sexually transmission	Symptomatic	High	Decreased
Kasprowicz *et al*[17]	37	Sexually transmission	Symptomatic	High	Irrespective
Rutebemberwa *et al*[62]	20	Injection drug use	Asymptomatic	High	Decreased

PD-1: Programmed death-1.

highly expressed on peripheral B cells and monocytes, since it is induced upon activation[56]. In patients with chronic HCV, $CD56^{high}$ NK cells expressed greater levels of PD-1, convenient considering their greater functional deficiency and less mature $CD56^{low}$ differentiation state[57]. In addition, PD-1 is expressed on Kupffer cells in the liver, other monocyte-derived cells, as well as epithelial, endothelial, and tumor cells[52,58,59].

Although HCV-specific $CD8^+$ T-cells are generally dysfunctional in HCV persistence, their level of impairment varied considerably among patients depending on PD-1 expression. Within an individual patient, the function and PD-1 expression of HCV-specific $CD8^+$ T-cells varied between the liver and peripheral blood[60]. Additional studies on the expression patterns of diverse splice variants of PD-1, PD-L1, and receptor-ligand interactions in diseased tissue will be important in determining a more comprehensive estimation of the level of the inhibitory signal and its effect on the outcome of human infection. Such studies will not only provide a superior mechanistic understanding of the PD-1 pathway in controlling T cell responses but will also encourage specific manipulation of this pathway therapeutically.

CORRELATION OF PD-1 EXPRESSION AND CLINICAL RESPONSES

The identification of cellular and molecular factors predicting clinical response to immunotherapy is strongly desirable, not only to aid in the design of therapies that overcome and enhance the inhibitory and stimulatory mechanisms, but also to preselect patients most likely to benefit from therapy and spare others from unnecessary exposure to possible side effects. Similar to its inhibitory role in anti-cancer immunity, the PD-1 signaling pathway has also been found to shape the overall immunity in HCV infection. For instance, PD-1 expression in acute HCV infection was found to be a signature of functional HCV-specific CD8 T cell exhaustion[16]. In this study, and as reported for the acute infection of HBV[61], the expression of PD-1 by HCV specific cytotoxic T cells was decreased in self-limited infections after the acute stage of infection in conjunction with $CD8^+$ T cell differentiation towards a memory CD127 phenotype[61]. In contrast, HCV specific $CD8^+$ T cells maintained high levels of PD-1 in patients with chronic advancement of infection and remained functionally impeded with no change from an effector to a memory phenotype[16]. Other studies, however, reported high levels of PD-1 expression (60%-100%) on all HCV specific $CD8^+$ and $CD4^+$ cells during the early stage of acute infection, irrespective of the clinical outcome or viral load[17]. Taken together, these results suggested that a role for the PD-1/PD-L1 interaction in regulating $CD8^+$ T cell function may exist under conditions of continuous high levels of HCV antigen stimulation.

Consistent with this suggestion, another study showed that the level of PD-1 expression in early phases of HCV infection was significantly more on HCV-specific T cells from patients who advanced to chronic HCV infection than from those who eliminated infection; and this correlation was independent of HCV RNA titer levels[62]. The reason for this difference is unclear, but it may be due to differences between the routes of infection between the two studies. This suggests that some of the biological differences that cause the development of symptoms likewise affect PD-1 expression. Additionally, the duration of infection in patients defined as acutely infected could be different between studies. Table 1 provides a list of studies involving PD-1 expression and the role of PD-1 in HCV infection.

PD-1 expression was investigated in 72 treatment-naïve patients with persistent HCV[57]. In this study, PD-1 expression was upregulated significantly not only on $CD4^+$ and $CD8^+$ T cells but also on NK cells, connecting with failed early and persistent virologic response to therapy. In contrast, patients with SVR demonstrated decreases in PD-1 after therapy completion, demonstrating that PD-1 expressed by NK cells is critical in persistent HCV[57].

$PD\text{-}1^+$ HCV-specific $CD8^+$ T cells in chronic HCV infection have a tendency to co-express Tim-3[63], 2B4, CD160 and other inhibitory molecules[64], particularly in the liver[65] since intrahepatic T cells showed a more exhausted phenotype than in the blood. In addition, the level of TIM-3 from patients with persistent HCV infection was greater than those who resolved the infection.

The results from other studies, however, are inconsistent regarding the differences in levels of PD-1/PD-L1 on HCV-specific $CD8^+$ cells contrasts between those who clear HCV infection and those with persistent infection[16,17,62]. Most of these studies though concluded that PD-1 expression levels are elevated on HCV-specific T cells *vs* naïve $CD8^+$ T cells or on T cells specific for some control antigens in the acute stage of infection, regardless of the outcome.

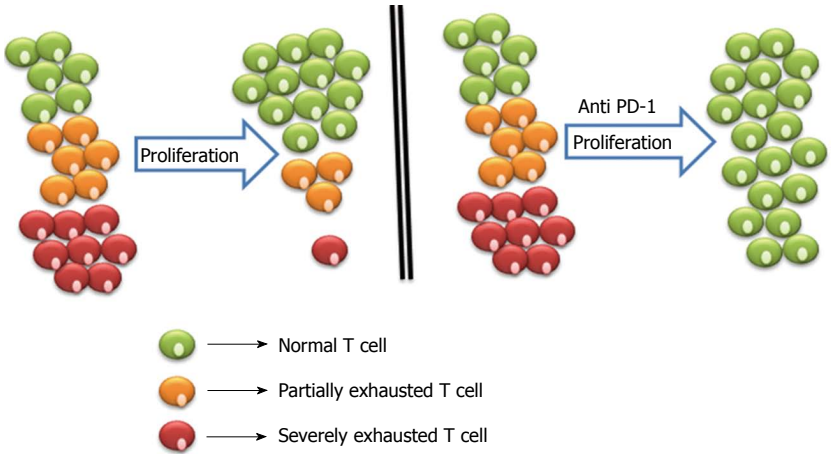

Figure 2 Proliferation of exhausted T cells and blockage strategies to reverse exhaustion. Severely exhausted T cells (red cells) proliferate poorly in comparison to partially exhausted and normal T cells (yellow and green cells). Antibody blockade of the pathway with PD-1 and its ligand reverses exhaustion and restores the functional capacities of exhausted T cells. PD-1: Programmed death-1.

RESTORATION OF ANTI-HCV RESPONSES *IN VITRO* BY BLOCKING PD-1

During the acute phase of HCV infection, HCV specific T cells have been characterized as poorly functional, regardless of the outcome of the disease[47-51]. A possible explanation for this behavior of the HCV-specific $CD8^+$ T response is exhaustion, which is supported by the initial rapid kinetics of HCV spread, replication after infection, and later on, by the continuous exposure of $CD8^+$ T cells to high antigen load. Several studies demonstrated that the PD-1/PD-L1 pathway plays a role in T-cell exhaustion when $CD8^+$ cells are chronically exposed to high level antigen loads. Blockade of this pathway can permit restoration of exhausted $CD8^+$ T cells[12,52-55] and results in expansion of HCV specific T cell proliferation[15,16,66] (Figure 2).

For instance, PD-L1 blockade improved HCV-specific T cell proliferation in a dose-dependent manner. However, proliferation of cytomegalovirus (CMV)-specific $CD8^+$ T cells was not affected by PD-1/PD-L1 blockade, consistent with their low expression levels of PD-1. Similar to its effects on $CD8^+$ T cells in HCV, blocking the PD-1/PD-L1 interaction *in vitro* restored effector function and enhanced the proliferative ability of exhausted $CD8^+$ T cells in many chronic infections, including HIV, HBV, simian immunodeficiency virus (SIV), LCMV, and Epstein-Barr virus (EBV)[13,15,67-71].

This functional T cell restoration by blocking the PD-1/PD-L1 pathway is a hierarchical phenomenon that appears to reflect the different sensitivities to exhaustion of the diverse T cell functions[72]. Restoration of proliferation capacity is relatively faster than the restoration of IFN-γ and IL-2 production but with no effect on cytotoxicity. In line with this, treatment with anti-PD-L1 antibodies does not always predict the expected positive effect of PD-1 blockade[73], suggesting that PD-L1 binds at least one additional receptor other than PD-1 to mediate its costimulatory function. Moreover, exhaustion was more effectively overcomed in HCV, than in HIV[68] by PD-1/PD-L1 blockade, as demonstrated by the increased capability of HCV-specific $CD8^+$ T cells to expand and to produce IFN and IL-2 after incubation with anti PD-L1 antibodies[15].

Collectively, these *in vitro* data suggest that PD-1 signaling on T cells is a significant inhibitory pathway during chronic HCV infection. Consequently, the possibility of partially restoring $CD8^+$ T cell function by blocking PD-1/PD-L1 interaction may provide a valuable tool for the enhancement of available therapies to cure chronic hepatitis C.

POTENTIAL USE OF *IN VIVO* PD-1 BLOCKING FOR ANTI-HCV THERAPY

As demonstrated above, *in vitro* blockade of PD-1 can restore the functionality of HCV-specific T cells. Blockade of PD-1 signaling was tested *in vivo* in both chimpanzees[74] and in patients with chronic HCV infection[75]. In the chimpanzee study, an increase in HCV specific $CD8^+$ cell responses and a considerable, although transient, reduction in HCV viremia was only seen in one of three chimpanzees. This chimpanzee had the strongest and broadest $CD4^+$ and $CD8^+$ T cell response before the development of chronic infection, which suggested that PD-1 blockade alone is not sufficient to attain viral clearance[74]. In the patient study, a single dose (10 mg/kg) of the PD-1 blocking antibody BMS-936558 was followed by a greater than 0.5 log10 IU/mL decrease in HCV RNA titer in five of 45 (11%) patients. At the highest dose given (10 mg/kg), a > 4 log10 IU/mL decrease in HCV RNA titer was seen in three of 20 (15%) patients. This decrease of HCV replication continued for more than 8 wk in most patients[75].

Interestingly, *in vivo* PD-L1 blockade does not appear to affect IL-10 level during chronic infection, although

it downregulated IL-10 and upregulated IL-2 and IFN-γ during *in vitro* stimulation[76,77]. Another *ex vivo* study showed that intrahepatic T cells were significantly dysfunctional and insusceptible to PD-1 blockade[60]. Therefore, the effect of PD-1 blockade is characterized by T cell compartmentalization. Additionally, PD-1 expression on HCV specific $CD8^+$ cells is affected by viral immune-evasion in chronic HCV infections[62].

Indeed, an efficient helper T cell function is compulsory for the development of virus specific CD8 cells[78]. Thus, the synergistic effect of CD8- and CD4-mediated T cell functions enhanced by anti-PD-L1 may represent a strategy to enhance the effect of available anti-HCV drugs. Consequently, it is conceivable that utilizing PD-1/PD-L1 blockade can enhance the antiviral effect of IFN/RBV therapy. Additional studies are required, however, to survey whether the enhancement of the T cell function induced by PD-1/PD-L1 blockade and favored by IFN-α therapy in patients with a recent HCV infection can be also accomplished in chronic infections of longer duration, where the effect of long lasting exhaustion may be more difficult to succeed. Therefore, the potential of partially restoring $CD8^+$ cells function by blocking PD-1/PD-L1 interaction could provide an additional tool to enhance available therapies to cure chronic HCV.

CONCLUSION

In spite of much progress in our understanding of T cell responses against HCV over the past decade, many critical research questions remain to be answered. It will be important to determine whether there is a causal correlation between the outcome of HCV infection and PD-1 expression. Functionally deficient exhausted HCV specific T cells are a significant cause and outcome of chronic viral infection. This is a great challenge to vaccine designs that either aim to eliminate or prevent such infections by interceding the T cell response. Whether developing a therapeutic vaccine to eliminate disease in infected patients or a prophylactic vaccine to prevent HCV in healthy individuals, it is imperative to keep this considerable obstacle in mind.

The possibility of restoring the function of HCV specific T cells by blocking the PD-1/PDL-1 pathway and reverting T-cell dysfunction in chronic HCV seems a worthy direction for anti-HCV immunotherapy. While the therapeutic application of this strategy in HCV infection is constrained by the recent, ongoing development of highly efficacious new treatments, it is promising that further investigation of PD-1 pathway blockade during antiviral therapies is warranted.

However, the systemic administration of PD-L1/PD-1 blocking antibodies carries the high risk of violating peripheral tolerance. Most tissues depend on PD-L1 expression to decrease T-cell effector activities that might cause autoimmune attack. To demonstrate this, knocking out PD-1 or PD-L1 pathways in mouse models causes severe, deadly autoimmunity[79,80]. In humans, single-nucleotide polymorphisms (SNP) of the *PDCD1* gene (encoding PD-1) are connected with systemic lupus erythematosus[81]. Therefore, efforts have to be made to enhance this promising strategy to maximize anti-HCV therapeutic activities while minimizing toxicity. We propose that one possible attractive alternative to the systemic blockade of PD-1 for HCV immunotherapy is to target the suppression of PD-1/PD-L1 co-stimulation during antigen presentation. This was demonstrated in mouse antigen presenting cells (APCs)[82,83]. This local and transient blockade may provide the positive effects needed to adequately boost anti-HCV immunity while restricting possible side effects to a minimum.

Overall, continuing work to understand better how the PD-1/PD-L1 pathway functions is imperative and will encourage development of new vaccination approaches that can overcome HCV specific T cell exhaustion.

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