JCI The Journal of Clinical Investigation

Unravelling the fate of functional PD1$^+$ T cells in chronic viral hepatitis

Eleanor Barnes

J Clin Invest. 2018;128(2):573-576. https://doi.org/10.1172/JCI99035.

Commentary

Hepatitis B virus (HBV) infection can be managed clinically with nucleos(t)ide therapy, which suppresses viral replication; however, these drugs must often be used long term, as they are unable to fully eliminate the virus. For many patients, discontinuation of treatment results in viral resurgence and hepatic flare, and there is not a reliable way to identify those individuals that can be successfully taken off nucleos(t)ide therapy. In this issue of the *JCI*, Rivino and colleagues report on their use of a multipronged approach to investigate potential biomarkers indicative of HBV-infected patients who can safely stop nucleos(t)ide therapy. The authors identified a population of HBV-specific, PD1-positive T cells that was present in HBV-infected patients who successfully discontinued treatment without hepatic flare, but not in those that developed flare upon treatment cessation. Together, these results support the concept that PD1$^+$ cells may play an important role in viral control, the further evaluation of this T cell subset in preventing hepatic flare, and the development of assays to better detect this PD1$^+$ T cell population in HBV-infected patients on nucleos(t)ide therapy.

Unravelling the fate of functional PD1+ T cells in chronic viral hepatitis

Eleanor Barnes

Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom (UK).

Hepatitis B virus (HBV) infection can be managed clinically with nucleos(t)ide therapy, which suppresses viral replication; however, these drugs must often be used long term, as they are unable to fully eliminate the virus. For many patients, discontinuation of treatment results in viral resurgence and hepatic flare, and there is not a reliable way to identify those individuals that can be successfully taken off nucleos(t)ide therapy. In this issue of the *JCI*, Rivino and colleagues report on their use of a multipronged approach to investigate potential biomarkers indicative of HBV-infected patients who can safely stop nucleos(t)ide therapy. The authors identified a population of HBV-specific, PD1-positive T cells that was present in HBV-infected patients who successfully discontinued treatment without hepatic flare, but not in those that developed flare upon treatment cessation. Together, these results support the concept that PD1+ cells may play an important role in viral control, the further evaluation of this T cell subset in preventing hepatic flare, and the development of assays to better detect this PD1+ T cell population in HBV-infected patients on nucleos(t)ide therapy.

The challenge of hepatitis B virus cure

Viral hepatitis remains a leading cause of death globally, with over 400 million people chronically infected with hepatitis B virus (HBV) (1) and an estimated 71 million infected with hepatitis C virus (HCV) (2). Left untreated, these infections can lead to progressive liver fibrosis, liver failure, and hepatocellular cancer. While HCV infection can now be cured using relatively short courses of oral therapies, HBV cure is rarely achieved, even after years of therapy; therefore, once initiated, treatment is usually maintained for the rest of the patient's life. The reason underlying the need for life-long treatment is that, although HBV nucleos(t)ide antiviral drugs inhibit viral replication, HBV covalently closed circular DNA (cccDNA), from which HBV mRNA transcripts are generated, remains intact within hepatocytes. Consequently, in the majority of cases, stopping HBV therapy results in a rapid reemergence of the virus that is often associated with liver inflammation and a detectable rise in liver transaminases, a phenomenon known as hepatic flares (3, 4). Hepatic flares may be associated with the development of liver fibrosis and hepatic decompensation, especially in those patients with underlying cirrhosis (5). Nevertheless, there is an important subset of patients in whom HBV antiviral therapy can be safely stopped. Currently, HBV surface antigen (HBsAg) seroconversion or HBsAg levels below 100 IU/ml, a level that likely represents the final stages of host immune control over HBV replication, are the only biomarkers used to identify patients who may be amenable to stopping nucleos(t)ide therapies (6). However, these low levels (or seroconversion) of HBsAg may take many years to achieve on therapy and are found in only a very small minority of patients. A thorough understanding as to why some people control HBV infection and others do not, which patients are on a path toward full immune control, and who will develop hepatic flares after cessation of HBV therapy are key questions that, if answered, will not only guide clinical management, but may give critical insights into HBV immune control that can be exploited for novel strategies of HBV immunotherapy.

In this issue, Rivino and colleagues have provided a comprehensive analysis that gets to the heart of these issues through the careful, longitudinal analysis of chronically HBV-infected patients before and after the cessation of HBV nucleos(t)ide antiviral therapy (7). In particular, this work focused on the identification of immune parameters associated with hepatic flare rather than long-term viral control (although these are linked to some extent) after stopping therapy. The study employed a combination of standard immunological techniques alongside cutting edge technologies to assess the functionality of both HBV-specific T cells and other relevant global immune cell populations. Rivino et al. employed tools, including IFN-γ ELISpot and flow cytometric assays, to measure HBV-specific T cell responses, mass cytometry (CyTOF) to assess global NK, T, and B cell populations, and NanoString technologies to evaluate the transcriptome of bulk CD4+ and CD8+ T cell subsets.

Prospective studies of HBV-infected patients conducted over several years may be required to assess treatment outcomes. Such a time frame represents a challenge in the HBV field, as these studies require both long-term funding and commitment from investigators. The work by Rivino et al. exemplifies why such commitment is required, as in this study, nucleos(t)ide therapy was withdrawn sequentially, with patients finally receiving lamivudine

▶ Related Article: p. 668

Conflict of interest: E. Barnes has patents pending for HCV and HBV vaccines and has recently partnered with Vaccitech (a spinout company from the University of Oxford) to develop immunotherapeutic HBV vaccines.
Reference information: *J Clin Invest*. 2018;128(2):573–576. https://doi.org/10.1172/JCI99035.

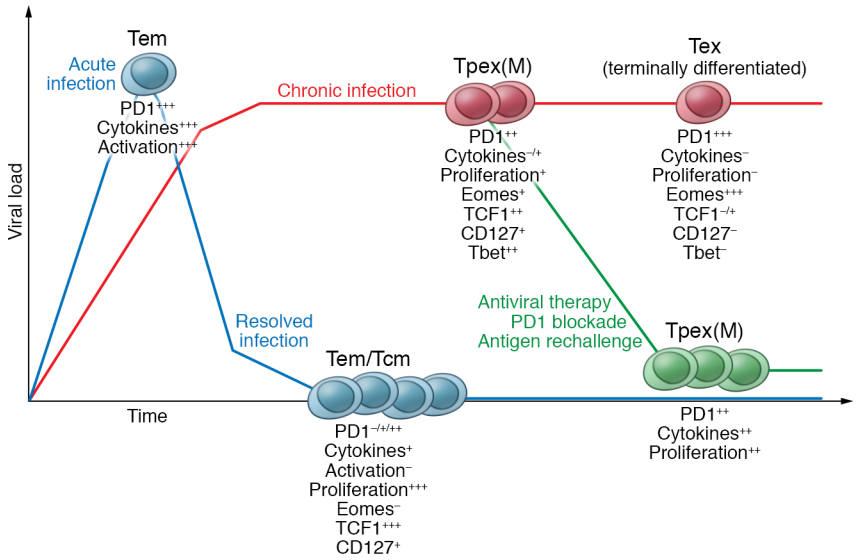

Figure 1. A schematic representation of PD1 expression and T cell differentiation in viral hepatitis. PD1 expression and selected discriminatory markers of T cell differentiation and the generation of T cell memory are shown during clinical stages of infection. These include acute and resolved viral infection (blue lines), chronic infection (red lines), and chronic infection with viral suppression with antiviral therapy (green lines). Partially exhausted T cells with memory potential have been shown to play a role in viral suppression during chronic disease in mouse models and can be expanded after therapy following in vivo or ex vivo antigen rechallenge. Tem, effector memory cells; Tcm, central memory cells; Tpex(M), partially exhausted T cells with memory potential.

monotherapy for 48 weeks before complete cessation of therapy. Approximately a third of the patients developed hepatic flares within 6 months after therapy was stopped, and these hepatic flares were associated with high levels of HBV DNA that were 100-fold greater than those observed in patients without flare.

Identifying markers of hepatic flare

Evaluation of the function and frequency of global NK, B, and T cells using CyTOF by Rivino et al. found no clear differences in these cell types between flare and nonflare patient groups. However, as it is well established that HBV-specific T cells associate with the control of HBV replication, HBV-specific T cells were evaluated before and after nucleos(t)ide therapy to determine whether this population could predict (and by implication causally contribute to) hepatic flares. Although HBV-specific responses were undetectable when measured ex vivo, these could be identified by IFN-γ ELISpot assays following stimulation with peptides across the whole HBV genome, with HBV-specific T cells targeting HBV core and polymerase antigens detected at significantly higher levels in the nonflare patients. Importantly, these findings suggest that hepatic flares are not driven by HBV-specific T cells, with the caveat that HBV-specific T cells were measured in the peripheral blood rather than in the liver where HBV-specific T cells ultimately will control replication. The study also suggests that HBV-specific T cells with proliferative capacity may be used to predict which patients can safely stop therapy and defines a specific cut-off for this purpose using a cultured ELISpot assay. However, HBV-targeting T cell detection with ELISpot is not a readily exportable technique, and there are simpler semiquantitative T cell assays, such as QuantiFERON assay, that may be more readily applied. Additionally, these observations suggest that HBV core and polymerase antigens may be particularly good targets for immunotherapeutic strategies.

Rivino et al. further evaluated factors that may contribute to hepatic flare by using NanoString technology to assess mRNA transcripts from more than 500 genes on flow cytometry–sorted $CD4^+$ and $CD8^+$ T cells. Unexpectedly, T cells from patients without flare showed an increase in transcripts that are conventionally associated with T cell exhaustion, including those encoding programmed cell death protein 1 (PD1) and T cell immunoglobulin domain (TIM3). Similar trends were observed in the CyTOF data, in which PD1 and TIM3 were increased in $CD8^+$ T cells. This intriguing result feeds into the current debate about the role of PD1 in chronic viral infections and raises the question of whether functional HBV-specific T cells could be recovered from $PD1^+$ populations. Rivino et al. used flow cytometry to sort peripheral blood mononuclear cells into $PD1^+$ and $PD1^-$ fractions and cultured these populations with core and polymerase HBV antigens. Interestingly, HBV-specific, cytokine-producing cells were best recovered from the $PD1^+$ populations and were enriched in nonflare patients. When bound to its ligand PDL1/2, PD1 suppressed T cell activation through the recruitment of SHP-2, which dephosphorylates and inactivates Zap70, a major integrator of T cell receptor–mediated signaling (8). The study by Rivino et al. implies that HBV-specific T cells control HBV replication and protect patients from hepatic flare when therapy is stopped; however, these functional cells appear to be enriched in $PD1^+$ T cell compartments, which are more traditionally associated with an exhausted T cell (Tex) phenotype.

Implications beyond HBV

The results of this work extend beyond the realm of HBV immunology and delve into the complexity that underlies the generation of T cell subsets during chronic viral infections in humans (Figure 1). Moreover, a better understanding of the T cell subsets linked to viral control may possibly be exploited for therapeutic gain. In particular, the findings of Rivino et al. are part of a growing body of work that challenges the conventional view (9) that PD1 expression on antiviral T cells is simply linked to and a marker of poorly functional Tex cells with little potential for recovery and manipulation. The remarkable results observed in some patients with cancer following the use of checkpoint modulators is perhaps the clearest evidence that T cells expressing PD1 can recover function (10). How-

ever, PD1 blockade is also associated with viral control in subsets of patients with chronic HBV and HCV infection (reviewed in ref. 11) and augments the effectiveness of therapeutic T cell vaccines in a woodchuck model of HBV infection (12).

Over the last decade, highly instructive studies using the mouse lymphocytic choriomeningitis virus (LCMV) model and extending into human studies of HBV, HCV, and HIV have shown that chronic infections are associated with Tex cells that express inhibitory molecules (including PD1), have poor proliferative capacity, exhibit a reduction in cytokine production, and have a specific transcriptional profile. Central to these observations is the concept that ongoing antigen exposure drives T cells to terminal differentiation and exhaustion; however, more recent data have shown this concept to be over simplistic. It appears that several subsets of Tex cells may coexist and are distinguished by the expression of transcription factors, with $Tbet^{hi}Eomes^{dim}$ $PD1^{intermediate}$ progenitor cells giving rise to terminally differentiated $Tbet^{dim}Eomes^{hi}$ $PD1^{hi}$ cells. Importantly, both Tex subsets are required to control infection (13). Furthermore, a subset of CXCR5- and TCF1-expressing $PD1^+$ cells with proliferative capacity and memory-like characteristics has been characterized using PD1 blockade in LCMV infection (14). Supporting these observations, studies in chronic HCV infection show that $CD127^+PD1^+$ HCV–specific T cells expressing TCF1 are maintained after antigen is removed with antiviral therapy and expand after viral relapse (15). PD1 expression is also maintained after spontaneous viral control in acute disease (16). Together, these studies identify $PD1^+$ T cell populations with memory potential in humans. The maintenance of the exhausted phenotype (defined as high PD1 and attenuated cytokine production) may be mediated by permanent epigenetic changes in the PD1-encoding gene (17) once antigen is removed. Arguably, the term exhausted should be reconsidered for PD1 high populations with memory-like features that contribute to viral control.

Implications and conclusions

The study by Rivino et al. was not able to directly address these $PD1^+$ T cell subsets, as HBV-specific T cells could not be characterized ex vivo. However, this study clearly adds to a growing literature that demonstrates that antiviral T memory cells with important functional characteristics may be derived from $PD1^+$ T cell populations in chronic infections. Elegant studies using the LCMV mouse model to evaluate the effects of genetic deletion of *Pd1* have taken these conclusions a step further and shown that PD1 expression is actually required for long-term maintenance of T cells in the face of ongoing antigen exposure, thereby preserving antiviral T cells from overstimulation and terminal differentiation (14). This process of memory preservation likely starts early after exposure to antigenic stimulation. This concept is nicely exemplified by human T cell vaccine studies, where PD1 is highly expressed soon after vaccination at the peak of the T cell response, followed by the subsequent generation of high-magnitude T cell memory (18).

In addition to giving new insights into immune control of HBV, several additional concepts have emerged from the data of Rivino et al. First, the observation that $PD1^+$ HBV–specific T cells remain partially functional, escape deletion, and provide antiviral function has implications for the use of checkpoint blockade in immunotherapeutic strategies for HBV that aim to cure patients. Potentially, the presence of these cells can be used to identify patients for which antiviral therapy can be safely stopped and may determine whether subsequent interventions, such as T cell vaccines, IFN therapy, checkpoint modulators, or a combination, should be applied. Ongoing studies to evaluate anti-PD1 therapy in patients with hepatocellular cancer suggest that these may be safely given to patients with HBV infection. Next, this study evaluated immune cells in peripheral blood, an approach that is appropriate for seeking biomarkers that may be clinically applied. However, our understanding of T cells subsets in the liver should be taken into consideration when interpreting the role of $PD1^+$ T cell subsets in viral control. Regardless of the underlying disease etiology, intrahepatic $CD4^+$ and $CD8^+$ T cells appear to be characterized by global upregulation of T cell inhibitory molecules, such as PD1 and 2B4 (19), supporting the hypothesis that intrahepatic T cells, which are constantly exposed to gut antigens and pathogens, must play a role in immune surveillance and regulation. Liver-resident memory T cells (Trm), which express high levels of both PD1 and CD39 (a combination that defines terminally exhausted Tex cells in HCV infection, ref. 20), have recently been shown to be expanded in patients with immune control of HBV infection. Critically, these cells are able to rapidly produce antiviral cytokines after polyclonal stimulation, an observation that supports the hypothesis that PD1 may preserve functional T cells within the hepatic environment (21).

Finally, this study, which centered on two relatively small patient cohorts, shows the potential utility of carefully phenotyping prospectively followed patients along with evaluating important clinical outcomes. This type of prospective clinical study should be supported and lies at the heart of an initiative in the UK, led by the Medical Research Council, to develop disease-based cohorts for stratified medicine studies, though as yet, HBV is not in the portfolio. The HBV field is ripe for such an approach in larger studies to properly account for patient heterogeneity.

Acknowledgments

E. Barnes is funded by the Medical Research Council (UK) as a Senior Clinical Fellow, leads the UK STOP-HCV consortium (http://www.stop-hcv.ox.ac.uk/home), and receives additional support from the Oxford National Institute for Health Research Biomedical Research Centre.

Address correspondence to: Eleanor Barnes, Peter Medawar Building for Pathogen Research, South Parks Rd, Oxford, OX1 3SY. Phone: 44.0.1865.281547; Email: ellie.barnes@ndm.ox.ac.uk.

1. Stanaway JD, et al. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. *Lancet*. 2016;388(10049):1081–1088.
2. World Health Organization. Global Hepatitis Report 2017. WHO Website. http://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/. Accessed December 12, 2017.
3. Papatheodoridis G, et al. Discontinuation of oral antivirals in chronic hepatitis B: a systematic review. *Hepatology*. 2016;63(5):1481–1492.
4. Liu Z, Liu F, Wang L, Liu Y, Zhang M, Li T. Clini-

cal characteristics and outcomes of patients with recurrent chronic hepatitis B after nucleos(t)ide analog withdrawal with stringent cessation criteria: A prospective cohort study. *Hepatol Res*. 2017;47(10):1000–1007.
5. Jeng WJ, et al. Off-therapy durability of response to entecavir therapy in hepatitis B e antigen-negative chronic hepatitis B patients. *Hepatology*. 2013;58(6):1888–1896.
6. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol*. 2017;67(2):370–398.
7. Rivino L, et al. Hepatitis B virus–specific T cells associate with viral control upon nucleos(t)ide–analogue therapy discontinuation. *J Clin Invest*. 2018;128(2):668–681.
8. Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, Saito T. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. *J Exp Med*. 2012;209(6):1201–1217.
9. Speiser DE, Utzschneider DT, Oberle SG, Münz C, Romero P, Zehn D. T cell differentiation in chronic infection and cancer: functional adaptation or exhaustion? *Nat Rev Immunol*. 2014;14(11):768–774.
10. Topalian SL, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366(26):2443–2454.
11. Wykes MN, Lewin SR. Immune checkpoint blockade in infectious diseases [published online ahead of print October 9, 2017]. *Nat Rev Immunol*. https://doi.org/10.1038/nri.2017.112.
12. Liu J, et al. Enhancing virus-specific immunity in vivo by combining therapeutic vaccination and PD-L1 blockade in chronic hepadnaviral infection. *PLoS Pathog*. 2014;10(1):e1003856.
13. Paley MA, et al. Progenitor and terminal subsets of CD8$^+$ T cells cooperate to contain chronic viral infection. *Science*. 2012;338(6111):1220–1225.
14. Odorizzi PM, Pauken KE, Paley MA, Sharpe A, Wherry EJ. Genetic absence of PD-1 promotes accumulation of terminally differentiated exhausted CD8$^+$ T cells. *J Exp Med*. 2015;212(7):1125–1137.
15. Wieland D, et al. TCF1$^+$ Hepatitis C virus-specific CD8$^+$ T cells are maintained after cessation of chronic antigen stimulation. *Nat Commun*. 2017;8:15050.
16. Kasprowicz V, et al. High level of PD-1 expression on hepatitis C virus (HCV)-specific CD8$^+$ and CD4$^+$ T cells during acute HCV infection, irrespective of clinical outcome. *J Virol*. 2008;82(6):3154–3160.
17. Youngblood B, et al. Chronic virus infection enforces demethylation of the locus that encodes PD-1 in antigen-specific CD8(+) T cells. *Immunity*. 2011;35(3):400–412.
18. Swadling L, et al. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. *Sci Transl Med*. 2014;6(261):261ra153.
19. Kroy DC, et al. Liver environment and HCV replication affect human T-cell phenotype and expression of inhibitory receptors. *Gastroenterology*. 2014;146(2):550–561.
20. Gupta PK, et al. CD39 Expression identifies terminally exhausted CD8$^+$ T cells. *PLoS Pathog*. 2015;11(10):e1005177.
21. Pallett LJ, et al. IL-2^{high} tissue-resident T cells in the human liver: Sentinels for hepatotropic infection. *J Exp Med*. 2017;214(6):1567–1580.