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T-cell therapy for chronic viral hepatitis

ANTONIO BERTOLETTI[1,2], ANTHONY TANOTO TAN[1] & SARENE KOH[2,3]

[1]*Program Emerging Infectious Diseases, Duke-NUS Graduate Medical School, Singapore,* [2]*Viral Hepatitis Laboratory, Singapore Institute for Clinical Sciences, A*STAR, Singapore, and* [3]*LION TCR pte, Singapore*

Abstract

Although therapy for chronic hepatitis C virus infection has delivered remarkable cure rates, curative therapies for hepatitis B virus (HBV) may only be available in the distant future. The possibility to eliminate or at least stably maintain low levels of HBV replication under the control of a functional anti-host response has stimulated the development of specific immunotherapies for HBV infection. We reviewed the development of T-cell therapy for HBV, highlighting its potential antiviral efficiency but also its potential toxicities in different groups of chronic HBV patients.

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are the only two communicable diseases in which there have been increases in related morbidity and mortality over the past 20 years [1]. Both viruses are chronically infecting about 500 million people (HBV ~350 million, HCV ~150 million) and represent the seventh most frequent cause of death worldwide [1]. HBV and HCV are hepatotropic, non-cytopathic viruses able to establish persistent infections that cause different degrees of hepatic inflammation (chronic hepatitis), leading to the development of liver cirrhosis and hepatocellular carcinoma (HCC).

The two viruses are unrelated and virologically different. HCV remains prevalent in North America and Europe, whereas chronic hepatitis B is prevalent in Asia and sub-Saharan Africa [1,2]. HCV is an RNA virus belonging to the *Flaviviridae* family, and HBV is a DNA virus of the *Hepadnaviridae* family and uses reverse transcriptase to synthesize its DNA from a pre-genomic RNA form [3]. HCV is able to activate in the infected host a classical type I interferon (IFN)-mediated innate response [3], whereas HBV generally escapes innate immune recognition and does not activate type I IFN-mediated immunity. Chronic HBV and HCV infections are both characterized by quantitative and functional defects of virus-specific T-cell response [4,5]. The frequency of virus-specific T cells is extremely low, and virus-specific T cells show features of exhaustion in both chronic HBV and HCV patients [6]. However, the quantitative and functional defects are more pronounced in HBV infections, with T cells virtually undetectable in the blood of many chronic HBV patients by *ex vivo* analysis [7–9]. In addition, while frequency and impact of viral mutations in T cell epitopes are frequently detectable in HCV infections [10], viral mutations affecting CD8 T-cell epitopes are scarcer in chronic HBV patients [6,11,12].

Of extreme practical importance in relation to the potential impact of T-cell therapy for HBV and HCV are the efficacies of currently available treatments. New therapies for HCV have delivered remarkable cure rates, with more than 90% of patients achieving viral clearance with all oral direct-acting antivirals [13]. In contrast, curative therapies for HBV will not be available until the distant future (14). Thus, although it is difficult to see a possible therapeutic advantage of a new T-cell-based therapy in chronic HCV patients, the fact that current therapies for HBV only partially suppress but do not eliminate HBV from the infected host has encouraged research for new and more radical therapies designed to eliminate or at least stably maintain low levels of HBV replication under the control of a functional anti-host response. For these reasons, in this review, we concentrate on the development of T-cell therapy for HBV. T-cell therapy for HCV chronic infection is certainly important for understanding the mechanisms of T-cell antiviral control [15,16], but their use for therapy appears unlikely.

Key Words: *HBV, HCV, immunotherapy*

The clinical need for T-cell therapy for chronic HBV infection

Current treatments for HBV include pegylated interferon alpha (Peg-IFNα) and nucleos(t)ide analogues (NAs), but neither are suitably efficient in providing a functional cure for chronic HBV infection [14]. Peg-IFNα can achieve sustained off-treatment control, but its success is limited to a small proportion of patients; approximately 10% of those treated achieve functional cure, defined as sustained loss of serum viremia and surface antigen of HBV (HBsAg) but not

Correspondence: **Antonio Bertoletti**, MD, Program Emerging Viral Diseases, Duke-NUS Graduate Medical School, 8 College Road, 169857 Singapore. E-mail: antonio@duke-nus.edu.sg

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complete HBV elimination because HBV always persists in few hepatocytes [17]. Peg-IFNα is primarily effective younger people with a moderate viral load. However, it has low applicability and is associated with side effects, leading to treatment discontinuation. In addition, Peg-IFNα can only be used in patients without contraindications to its use, such as those with mild/moderate disease/compensated cirrhosis, and not in contraindicated in decompensated patients with cirrhosis. Although NAs sufficiently suppress the production of new virions, reducing HBV DNA to undetectable levels in the serum and normalizing transaminases, which may in turn reduce fibrosis and the development of cirrhosis, HBsAg loss is rarely achieved. NAs directly target HBV DNA synthesis only and thus are ineffective in eradication of the covalently closed circular DNA (cccDNA), the episomal form of HBV from infected cells [18]. Treatment with NAs is thus considered lifelong, but this has potential risk of long-term toxicity [19], with limited data on treatment discontinuation; this results in reactivation of HBV in the majority of patients. For these reasons, new curative therapeutic options for chronic HBV infection are urgently needed, and the strategies can be divided into those that directly target HBV replication or those that target the host immune system—in particular, T cells that are considered essential for HBV control.

Why use T cell therapy in chronic HBV infection

The importance of T cells in establishing a functional cure of chronic HBV infection is derived from data obtained in patients and animal models. In chimpanzees acutely infected with HBV, deletion of CD8 T cells causes chronic HBV infection [20]. Patients with acute HBV infection mount a cellular anti-HBV immune response that is temporally correlated with serum HBV clearance [21–24]. However, HBV-specific T cells are quantitatively and functionally defective in chronic HBV patients [6]. On the other hand, immunosuppressive treatments targeting cellular immunity in anti-HBV core-positive subjects often trigger rapid HBV reactivation [25]. These data therefore indicate that virus-specific T cells have the capacity to maintain HBV infection under tight replicative control.

As a direct consequence, immunotherapies designed to augment HBV-specific T-cell responses in chronic HBV patients might achieve the level of HBV control present in subjects who resolve acute infection, who may not fully eradicate HBV, as the covalently closed circular DNA (cccDNA) can remain in some hepatocytes [17,26]. However, HBV antigens and DNA are undetectable, and their risk of developing severe liver pathology, such as HCC, is extremely low [26]. The therapeutic strategies designed to boost HBV-specific T-cell responses in chronic HBV patients can be divided into strategies designed to boost the defective HBV-specific T cells still present in some chronic HBV patients or the ones designed to produce new HBV-specific T cells that can be adoptively transferred in patients (Figure 1).

Therapies based on the concept of boosting HBV-specific T cells present in limited numbers in patients with chronic HBV infection are primarily represented by therapies with checkpoint inhibitors (anti-PD-1, anti-CTLA-4, etc.) or therapeutic vaccines. Experimental data have shown that the function of peripheral and intrahepatic exhausted HBV-specific T cells [27,28] can be partially restored *in vitro* by

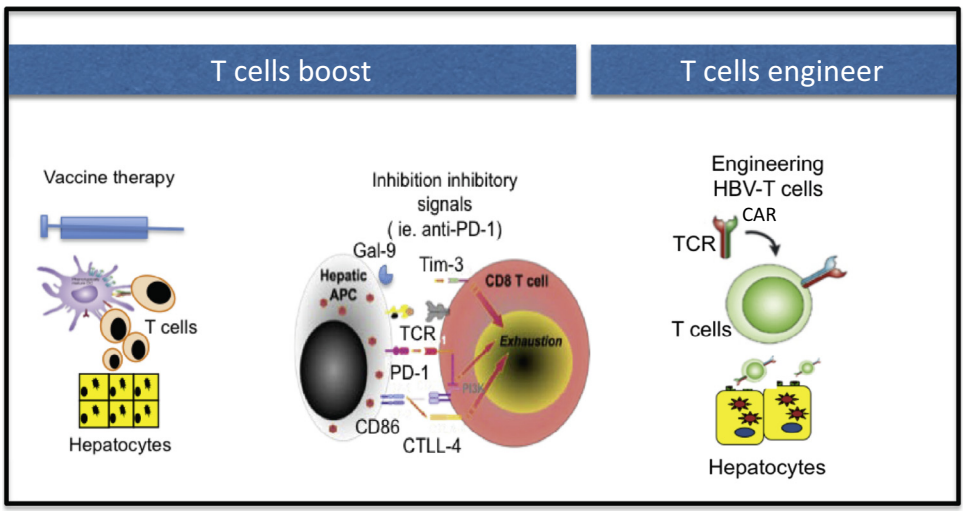

Figure 1. Strategies to boost HBV-specific T cells.

anti-PD1/PDL-1 blockade. However, at present, therapies with checkpoint inhibitors have been used with success only in some solid malignancies such as lung cancer, renal cell carcinoma and melanoma [29,30], with limited data in HCC and reports of only limited efficacy in chronic HCV patients [31]. No data are available thus far in chronic HBV patients.

On the other hand, vaccine therapies have already been used in several trials in chronic HBV patients. Attempts to boost the quantity and function of antiviral T cells using different HBV vaccine compositions or activation of professional antigen presenting cells naturally loaded with HBV antigens [32] have shown some efficacy *in vitro* and in animal models [33]. However, initial trials with the use of classical prophylactic vaccines show suboptimal and conflicting results [34–36]. Moreover, attempts to use new vaccine formulations or combination therapies with antivirals have demonstrated only limited effect [37–39].

The extreme rarity of HBV-specific immune cells in chronic HBV patients, their exhausted phenotype [5,27,40,41], metabolic alterations [42,43] and in consequence the difficulty in boosting HBV-specific immunity have, however, stimulated the development of strategies of immune restoration based on adoptive transfer of engineered HBV-specific T cells [44]. The rationale of modified T-cell therapy is centered on the concept that HBV-specific T cells in patients with chronic HBV infection are so heavily compromised (quantitatively, functionally, and metabolically) or even deleted due to lifelong contact with viral antigens presented by hepatocytes, that their rescue through checkpoint inhibitors or vaccines would be unlikely, and thus interventions to directly replenish the deleted virus-specific T-cell repertoire are required.

Furthermore, clinical evidences have suggested that adoptive transfer of HBV-specific T cells might lead to successful HBV control. For example, treatment of patients with leukemia and chronic HBV infection with bone marrow transplant from subjects with an HBV-specific T-cell response (subjects vaccinated with HBV or subjects who have spontaneously controlled HBV infection) [45,46] leads to HBV clearance. Similarly, transplantation of an HBV-positive liver in a subject with HBV-specific adaptive immunity resulted in subsequent HBV clearance [47] associated with an increase in HBV-specific cellular and humoral responses. Taken together, these data support the hypothesis that direct adoptive transfer of HBV-specific T cells in patients with chronic HBV infection might lead to HBV control.

For these reasons, design and expansion of different engineered HBV-specific T cells for adoptive T-cell transfer have been attempted. T cells able to recognize HBV-infected cells have been constructed using a chimeric antigen receptor made of an anti-HBV-specific antibody or using canonical human leukocyte antigen class I restricted HBV-specific T-cell receptors [48–50]. These cells are able to recognize HBV-infected targets *in vitro* while data in animal models [50] or in selected clinical situations [51] have been encouraging, showing the ability of these cells to recognize HBV-expressing hepatocytes or HCC cells with HBV DNA integrations *in vivo* and their ability to induce a substantial reduction of HBsAg. Furthermore, new unpublished data in humanized chimeric mice carrying HBV-infected human hepatocytes demonstrated the ability of T-cell receptor (TCR)-redirected T cells to recognize HBV-infected hepatocytes and cause a significant drop of HBV DNA (Kah *et al.*, manuscript in revision).

A major problem of adoptive T-cell therapy is the practical difficulty in implementing them for clinical use [52]. The production of large quantity of engineered T cells is still cumbersome, strictly regulated and necessitates a laboratory with highly skilled personnel. New technology and methods are likely to progressively overcome these practical problems, but at present, such therapies remain possible in only a few laboratories [53].

For these reasons, strategies that use antibodies with TCR-like specificities [54] or soluble TCR [55] able to redirect endogenous T cells against their targets have been proposed. The advantages of these strategies are that they do not require manipulation of T cells outside the patient's body. The antibodies or the soluble TCR, designed to induce specific accumulation and triggering of endogenous T cells in close contact with their specific targets, can be injected directly to the patients. Currently such a strategy has been used with success in HIV infection (soluble TCR) [56], whereas TCR-like antibodies have only been used to deliver cytokine payloads directly toward HBV-infected targets [57].

Controlling hepatic toxicity in HBV-specific T-cell therapy

Therapeutic strategies designed to boost HBV-specific T cells in patients with chronic HBV infection carry the inherent risk of inducing severe liver inflammatory events and because the liver is an organ indispensable for life, such risk needs to be carefully managed.

HBV is a non-cytopathic virus; CD8 T cells are essential for viral control, but in addition to lysing HBV-infected hepatocytes, they can trigger liver inflammatory events [58,59]. It is, however, difficult to predict the extent of liver damage that a specific number of HBV-specific CD8 T cells can induce.

Liver inflammation is related not only to the number and fitness of restored HBV-specific CD8 T cells (i.e., by checkpoint inhibitor therapy) or adoptively transferred engineered HBV-specific T cells but also to the modification of the liver environment induced by the chronic infection. Human studies performed to quantify the number of HBV-specific CD8 T cells in different clinical situations have, for example, clearly demonstrated that a direct proportionality between HBV-specific CD8 T cells and extent of liver damage is observed only in acute hepatitis patients [23]. Such direct proportion does not exist in chronic HBV patients where liver damage is related to the ability of HBV-specific CD8 T cells to trigger the recruitment of inflammatory cells [60]. Furthermore, it has recently been shown that similar number of HBV-specific T cells can be demonstrated in chronic HBV patients with active hepatitis or in those with a complete absence of liver inflammatory events [61,62]. The causes of these different liver inflammatory patterns are still not completely understood, but many other variables have been shown to modulate liver inflammatory events and T-cell fitness. For example, release of arginase directly from hepatocytes or from myeloid suppressor cells has been shown to dampen T-cell function [63–65]. Equally, NK cells [66] or regulatory cells [67] present in the liver might inhibit T-cell function or suppress inflammatory events, whereas chronic liver inflammation might block the access of T cells to their targets by altering the normal anatomy of liver endothelial cells. On the other hand, liver inflammatory events can profoundly alter the population of intrahepatic myeloid cells. The recruitment of inflammatory monocytes might alter the normal liver tolerogenic environment, characterized by the presence of Kupffer cells with tolerogenic and anti-inflammatory features [68], to a more pro-inflammatory environment. Similar levels of CD8 T-cell response in different liver microenvironments would trigger different degree of liver inflammation (Figure 2).

To reduce the risk of inducing severe liver damage, research efforts have been focused on developing T cells with reduced ability to trigger inflammatory events. Indeed, if the restoration of HBV-specific T-cell response in chronic HBV patients through checkpoint inhibitors or vaccines is difficult to modify or predict and would be related to the quantity of exhausted T cells present in the distinct groups of patients, adoptive T-cell transfer can be performed with engineered HBV-specific CD8 T cells with reduced ability to trigger inflammatory events.

One of these strategies is based on the use of T cells engineered to transiently express HBV-specific TCRs through nonviral gene transfer systems. T cells transduced by viral vectors stably express the

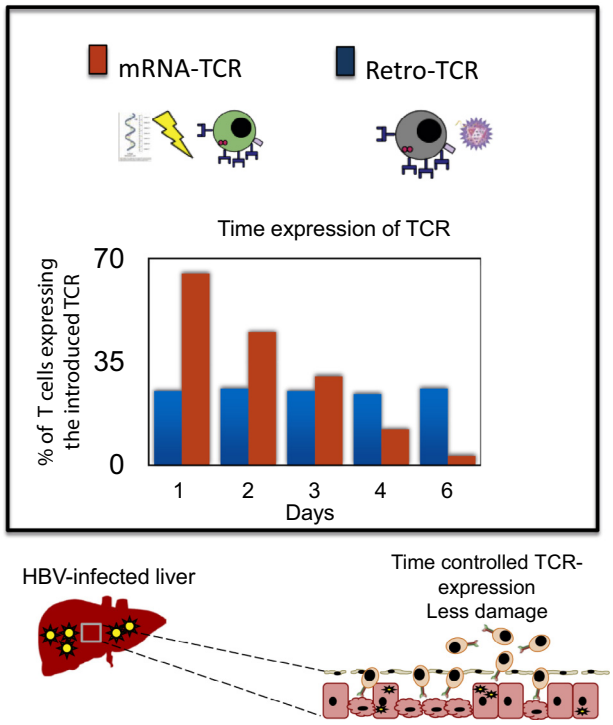

Figure 2. TCR expression on mRNA TCR electroporated T cells is transient. This results in a TCR expression limited by time and a reduced chronic liver damage.

TCRs, and their unchecked expansion might lead to progressive liver toxicities that is difficult to manage clinically. To bypass this problem, we implemented a strategy in which T cells are engineered through messenger RNA (mRNA) electroporation, and they express HBV-specific TCRs transiently for only 3–5 days (Figure 2) [69]. In addition, to circumvent safety concerns related to stable genetic manipulation of T cells induced by viral vectors, these cells with limited life span can be adoptively transferred in escalating doses, and their potential toxicities are more easily managed than stably transduced TCR-redirected T cells. Despite their transient expression of TCRs, mRNA TCR-redirected T cells control the expansion of HBV-expressing hepatoma cells in mice [69]. Furthermore, the antiviral activity of mRNA TCR-redirected T cells was also recently tested in an HBV-infected human liver chimeric uPA/SCID/ILγR2 (USG) mouse model. These mRNA TCR-redirected T cells were shown to preferentially home to the liver of HBV-infected mice and induced a progressive but timely controlled virus-specific immune-mediated reduction of serological and intrahepatic HBV viral loads (~1–1.5 log reduction of HBV-DNA after three doses of 0.5 million T cells).

It is important to point out that the antiviral efficacy of mRNA TCR-redirected T cells was already detectable 4 days after T-cell infusion and multiple

injections (n = 3) were able to induce progressive decrease of viremia (median 1 log) in only 12 days (Kah *et al.*, manuscript in preparation). Such rapid reduction of viremia in high viremic humanized mice is quite remarkable and comparable to the kinetics of viremia reduction often achieved in this model using approved antiviral drugs such as polymerase inhibitors (NAs therapy) or IFN-alpha [70]. Most importantly, mRNA TCR-redirected T cells trigger only a temporary immune-mediated killing of the infected hepatocytes 8–10 days after infusion and the treated mice show a reversion of transaminase (an enzyme released by dying hepatocytes) levels identical to the ones detected before therapy. Such data are important for the clinical translation of T-cell therapy into patients because the temporary effect of mRNA TCR-redirected T cells will allow a safe implementation of dose escalating regimes. This could allow the optimization of dose and therapy length required to achieve substantial reduction of HBV-infected hepatocytes pool without triggering severe inflammatory events that should subside when the expression of HBV-specific TCRs disappear from the adoptively transferred T cells. The flexibility of such cell preparation will also allow the study of possible combination therapy with drugs suppressing HBV replication (NAs) or blocking hepatocyte reinfection and intrahepatic HBV spreading (Myrcludex-B) [71].

Production of antiviral non-cytolytic HBV-specific T cells

The immunological control of HBV and HCV viruses does not necessarily require the direct lysis of infected hepatocytes but can also be achieved through non-cytopathic mechanisms triggered by antiviral cytokines [72,73]. In addition, up-regulation of the nuclear APOBEC3 cytidine deaminases A3A, A3B and A3G in HBV-infected hepatocytes through interferon-α and lymphotoxin-β-receptor activation have been shown to inhibit HBV replication by the induction of HBV-DNA hyper-mutations [74–76] and even possibly degrade cccDNA without causing hepatotoxicity [77]. Following this line of thought, research efforts have been channeled to produce HBV-specific TCR-redirected T cells able to inhibit HBV replication with limited lytic and pro-inflammatory activity. Two strategies have been proposed. One possibility is to down-regulate the production of Granzyme B and perforin on activated T cells with specific anti-sense oligonucleotides and thus produce HBV-specific TCR-redirected T cells with low lytic ability to be used for adoptive T-cell transfer. The other alternative is to exploit one characteristic of mRNA electroporation that, in contrast to viral vectors, can also be applied to non-dividing cells. This could allow the introduction

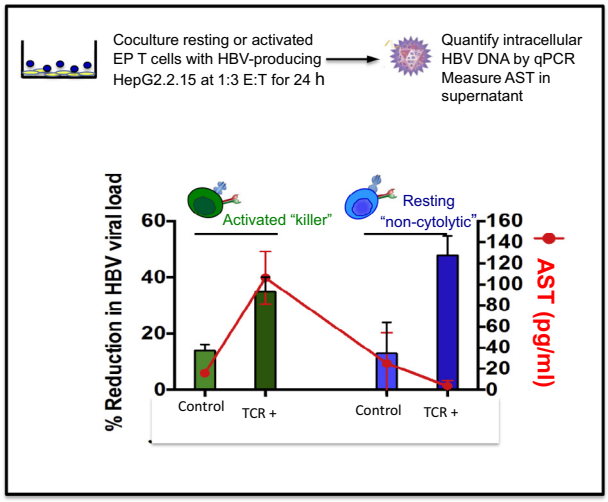

Figure 3. Resting TCR-redirected T cells inhibit HBV replication without lysis. AST, Alanine transaminases; EP, electroporated T cells; qPCR, quantitative polymerase chain reaction.

of TCR-mRNA in non-activated resting primary human T cells, which have lower Granzyme B and perforin contents than activated T cells [78]. Preliminary results have shown that it is possible to engineer non-cytolytic human T cells that suppress HBV and HCV replication without overt hepatotoxicity and pro-inflammatory cytokine release (Figure 3) (Koh *et al.*, manuscript in preparation). Inhibition of viral replication was mediated not only by release of IFN-gamma but also by activation of the APOBEC3 pathway in HBV-infected cells. These "resting mRNA-TCR electroporated T cells" will need to be first tested in animal models but could present a novel immunotherapeutic strategy to reduce the bulk of viral-infected hepatocytes without causing severe liver damage.

Conclusions

In this short review, we have summarized why T-cell therapy in chronic HBV infection has a strong rationale and could potentially achieve sustained HBV control in most patients. We must, however, point out that although these immune therapies currently in the pipeline show promise, their efficacy in the real-world setting remains to be seen. At the moment, immune therapies based on vaccines have failed in humans, and data with anti-PD1 in patients with HBV-related HCC have also shown sparse results in terms of HBV control.

We have recently argued (Upkar Gill and Bertoletti, *Seminars in Liver Disease* [79]) that the low efficacy of such new immune therapeutic interventions might be dependent on the criteria of chronic HBV patients selection, that until today, have selected only adult

chronic HBV patients with chronic active hepatitis. New T-cell therapies designed to boost exhausted virus-specific T cells might be better suited for treating young patients in the so-called immune-tolerant phase of disease, more so than older subjects with chronic active hepatitis B. In immunological terms, younger subjects have less compromised HBV-specific T-cell function and a lower "pro-inflammatory response" than older subjects; thus, they might be more likely to recover HBV-specific T-cell immunity and less likely develop a severe inflammatory liver reaction (see also [80,81]). Similar arguments can be used for therapies based on adoptive transfer of engineered T cells that are likely to be more efficient and less dangerous in a liver environment devoid of inflammatory infiltrate. Selecting chronic HBV patients for these novel T-cell therapies will be challenging and will likely require the acceptance of new paradigms of patients selection. We are however, confident that the potential efficacy of T-cell therapy for the cure of chronic HBV infection will further stimulate research in this field and allow immunotherapy to be considered a real therapeutic option in chronic HBV infection.

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