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HBsAg-redirected T cells exhibit antiviral activity in HBV-infected human liver chimeric mice

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Abstract

Background. Chronic hepatitis B virus (HBV) infection remains incurable. Although HBsAg-specific chimeric antigen receptor (HBsAg-CAR) T cells have been generated, they have not been tested in animal models with authentic HBV infection. *Methods.* We generated a novel CAR targeting HBsAg and evaluated its ability to recognize HBV+ cell lines and HBsAg particles *in vitro*. *In vivo*, we tested whether human HBsAg-CAR T cells would have efficacy against HBV-infected hepatocytes in human liver chimeric mice. *Results.* HBsAg-CAR T cells recognized HBV-positive cell lines and HBsAg particles *in vitro* as judged by cytokine production. However, HBsAg-CAR T cells did not kill HBV-positive cell lines in cytotoxicity assays. Adoptive transfer of HBsAg-CAR T cells into HBV-infected humanized mice resulted in accumulation within the liver and a significant decrease in plasma HBsAg and HBV-DNA levels compared with control mice. Notably, the fraction of HBV core–positive hepatocytes among total human hepatocytes was greatly reduced after HBsAg-CAR T cell treatment, pointing to noncytopathic viral clearance. In agreement, changes in surrogate human plasma albumin levels were not significantly different between treatment and control groups. *Conclusions.* HBsAg-CAR T cells have anti-HBV activity in an authentic preclinical HBV infection model. Our results warrant further preclinical exploration of HBsAg-CAR T cells as immunotherapy for HBV.

Key Words: *adoptive immunotherapy, CAR T cells, hepatitis B virus*

Introduction

Hepatitis B virus (HBV) is a global pandemic chronically infecting 300 million people across the world today [1]. In these chronic patients, HBV causes a lifelong infection that can lead to liver cirrhosis or cancer in 25% of patients [2]. HBV therapies currently remain limited to reverse transcriptase inhibitors (RTIs) and interferon (IFN)-α. RTIs only suppress HBV-DNA levels without significantly affecting the transcriptional template, covalently closed circular DNA (cccDNA) [3], and IFN-α causes significant side effects with little long-term therapeutic benefit [4]. Thus, new anti-HBV therapies are urgently needed to cure the infection.

During the HBV-specific immune response in acutely resolving patients, infiltrating T cells rapidly purge the liver of HBV [5,6]. In chronic HBV patients, however, HBV-specific T cells are present only in low frequency and/or are anergic [7]. CD8-positive T cells have been shown to be crucial in resolution of acute HBV infection [6]. They are able to clear HBV in both cytolytic and noncytolytic effector functions [8]. The cytokines IFN-γ and tumor necrosis factor

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(TNF)-α, released by T cells, are important in driving noncytolytic suppression of virus [9,10]. Both IFN-γ and TNF-α can induce degradation of intracellular cccDNA [11], explaining part of this mechanism.

The adoptive transfer of T cells genetically engineered to target hepatitis B surface antigen (HBsAg) on the surface of infected hepatocytes [12] with chimeric antigen receptors (HBsAg-CAR T cells) is an attractive strategy to reconstitute HBV-specific T cell immunity. Indeed, HBsAg-CAR T cells have been shown to eliminate cccDNA from HBV-infected primary hepatocytes *in vitro* [13] and to have transient anti-HBV activity in a transgenic HBV mouse model [14]. The HBV transgenic mouse model harbors an integrated copy of the HBV genome [15], is born with tolerance to viral antigens and lacks cccDNA formation. Thus, transgenic HBV mice can only model viral suppression and not complete T cell-mediated cure. Given that HBV naturally infects only humans and chimpanzees at high levels [16], finding appropriate models to test cure strategies is challenging. With previous testing only in transgenic mice, it remains an open question whether HBsAg-CAR T cells can induce a reduction of HBV levels in a model with authentic infection harboring episomal HBV cccDNA. Here we address this question by evaluating human HBsAg-CAR T cells in HBV-infected human liver chimeric mice. These mice are immunodeficient and repopulated with human hepatocytes [17,18], allowing for spreading infection with HBV entry and cccDNA formation [19]. Thus, this model closely mimics HBV infection and is ideal to test the ability of HBsAg-CAR T cells to eradicate HBV genomes and/or infected hepatocytes.

Methods

Generation of retroviral vectors encoding CARs

To generate pSFG-HBs-G4m-28-zeta, a scFv encoding the amino acid sequence VH and VL domains of the XTL-19 antibody (mAb 19.79.5) [20] termed HBs was synthesized (IDTDNA, Coralville, IA), and cloned into an SFG retroviral vector using 5′ NcoI and 3′ BamHI, replacing the antigen binding domain from a second generation CAR vector, IL13Rα2-hIgG1-CD28-zeta CAR [21]. Next, the hIgG1 hinge was replaced with a mini-gene (synthesized by IDTDNA, Coralville, IA) encoding the CH2-CH3 domain from human IgG4 with mutated Fc receptor binding sites [22] (G4m) to generate pSFG-HBs-G4m-28-zeta. To generate pSFG-Ctrl-G4m-28-zeta, the HBs-specific scFv in pSFG-HBs-G4m-28-zeta was replaced by polymerase chain reaction (PCR) cloning with an scFv specific for EGFRvIII [23]. pSFG-HBs-CH3-28-zeta was generated by cloning the HBs-specific scFv into the 5′ NcoI and 3′ BamHI sites of a pSFG vector with a CAR.IgG1-CH3.28-zeta expression cassette (gift of Dr. Maksim Mamonkin, Baylor College of Medicine, Houston, TX). Cloning was verified by sequencing (Lone Star Labs). RD114-pseudotyped retroviral particles were generated by transient transfection of 293T cells as previously described [24].

Generation of CAR T cells

To generate CAR T cells, PBMCs were isolated by Lymphoprep (Greiner Bio-One) gradient centrifugation and then stimulated on treated non-tissue-culture 24-well plates, which were pre-coated with OKT3 (CRL-8001, ATCC) and CD28 (BD Bioscience) antibodies. Recombinant human interleukin (IL)-7 and IL-15 (IL-7, 10 ng/mL; IL-15, 5 ng/mL; PeproTech) were added to cultures on day 2. On day 3, OKT3/CD28 stimulated T cells (2.5×10^5 cells/well) were transduced on RetroNectin (Clontech)-coated plates in the presence of IL-7 and IL-15. On day 5 or 6, T cells were transferred into tissue culture plates and subsequently expanded with IL-7 and IL-15. CAR expression was determined 4–5 days post-transduction by FACS analysis using a human IgG (H + L) antibody (Jackson ImmunoResearch), and appropriate isotype control.

Cell culture assays

HepG2.2.15 cells (gift of Dr. Betty Slagle, Baylor College of Medicine, Houston, TX) and HepG2 cells (ATCC) were cultured in Dulbecco's Minimal Essential Media supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic (Gibco ThermoFisher). For co-culture assays, 1 million HepG2 or HepG2.2.15 cells were plated; 24 h later, cells were washed, and 2 million T cells were added in one well of a 24-well plate in duplicate without exogenous cytokines. After 24 h, supernatant was removed to assess cytokine release. For cytokine ELISAs, concentrations of INF-γ were assessed using manufacturer's protocol (R&D Systems). For IL-2 and TNF-α, cytokine levels were determined using a multiplex assay (Millipore). For cytotoxicity assays, standard chromium release protocols were followed as previously described [21] using the same 2:1 ratio of T cell–to–target-cell, wherein target cells were plated on the same day as T cell addition preventing any HBsAg particle accumulation. Cell culture assays were repeated with at least three donors.

For cytokine assays based on co-culture with HBsAg particles, the HepG2.2.15 supernatant was collected (measured to be 80 ng/mL HBsAg using ELISA protocol described subsequently). Culture media contained 50% HepG2.2.15 supernatant with 1 million CAR T cells. At 24 h, supernatant was collected for use in cytokine ELISA assays and electron microscopy