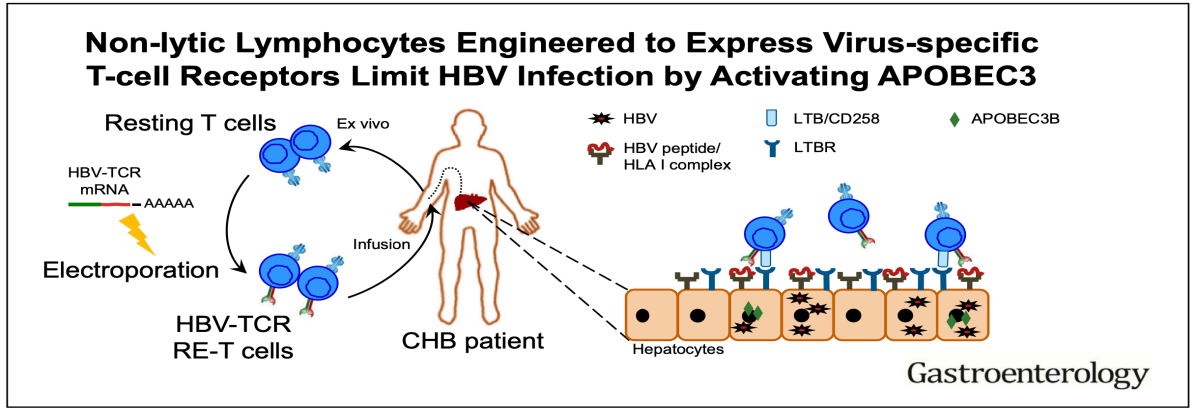
Gastroenterology 2018;155:180–193

BASIC AND TRANSLATIONAL—LIVER

Nonlytic Lymphocytes Engineered to Express Virus-Specific T-Cell Receptors Limit HBV Infection by Activating APOBEC3

Sarene Koh,[1,2] Janine Kah,[3] Christine Y. L. Tham,[1,4] Ninghan Yang,[1] Erica Ceccarello,[5,6] Adeline Chia,[7] Margaret Chen,[8] Atefeh Khakpoor,[7] Andrea Pavesi,[6] Anthony T. Tan,[7] Maura Dandri,[3,9] and Antonio Bertoletti[1,7]

*[1]Singapore Institute for Clinical Sciences, Agency for Science, Technology and Research (A*STAR), Singapore; [2]Lion TCR Private Limited Singapore, Singapore; [3]Department of Internal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [4]NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore; [5]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [6]Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A*STAR), Singapore; [7]Emerging Infectious Diseases (EID) Program, Duke-NUS Medical School, Singapore; [8]Department of Dental Medicine, Karolinska Institutet, Sweden; [9]German Center for Infection Research, Hamburg-Lübeck-Borstel Partner Site, Germany*



0016-5085/$36.00
https://doi.org/10.1053/j.gastro.2018.03.027

Abstract

BACKGROUND & AIMS: Strategies to develop virus-specific T cells against hepatic viral infections have been hindered by safety concerns. We engineered nonlytic human T cells to suppress replication of hepatitis B virus (HBV) and hepatitis C virus (HCV) without overt hepatotoxicity and investigated their antiviral activity.

METHODS: We electroporated resting T cells or T cells activated by anti-CD3 with mRNAs encoding HBV or HCV-specific T-cell receptors (TCRs) to create 2 populations of TCR-reprogrammed T cells. We tested their ability to suppress HBV or HCV replication without lysis in 2- dimensional and 3-dimensional cultures of HepG2.2.15 cells and HBV-infected HepG2-hNTCP cells. We also injected TCR-reprogrammed resting and activated T cells into HBV- infected urokinase-type plasminogen activator/severe combined immunodeficiency disease/interleukin 2g mice with humanized livers and measured levels of intrahepatic and serological viral parameters and serum alanine aminotransferase. Livers were collected for analysis of gene expression patterns to determine effects of the TCR-reprogrammed T cells.

RESULTS: TCR-reprogrammed resting T cells produced comparable levels of interferon gamma but lower levels of perforin and granzyme than activated T cells and did not lyse HCV- or HBV-infected hepatoma cells. Although T-cell secretion of interferon gamma was required to inhibit HCV replication, the HBV- specific TCR-reprogrammed resting T cells reduced HBV replication also through intracellular activation of apolipoprotein B mRNA editing enzyme, catalytic polypeptide 3 (APOBEC3). The mechanism of APOBEC3 intracellular activation involved temporal expression of lymphotoxin-b receptor ligands on resting T cells after TCR-mediated antigen recognition and activation of lymphotoxin-b receptor in infected cells.

CONCLUSIONS: We developed TCR-reprogrammed nonlytic T cells capable of activating APOBEC3 in hepatoma cells and in HBV-infected human hepatocytes in mice, limiting viral infection. These cells with limited hepatotoxicity might be developed for treatment of chronic HBV infection.

TCR mRNA Electroporation in RE-T Cells

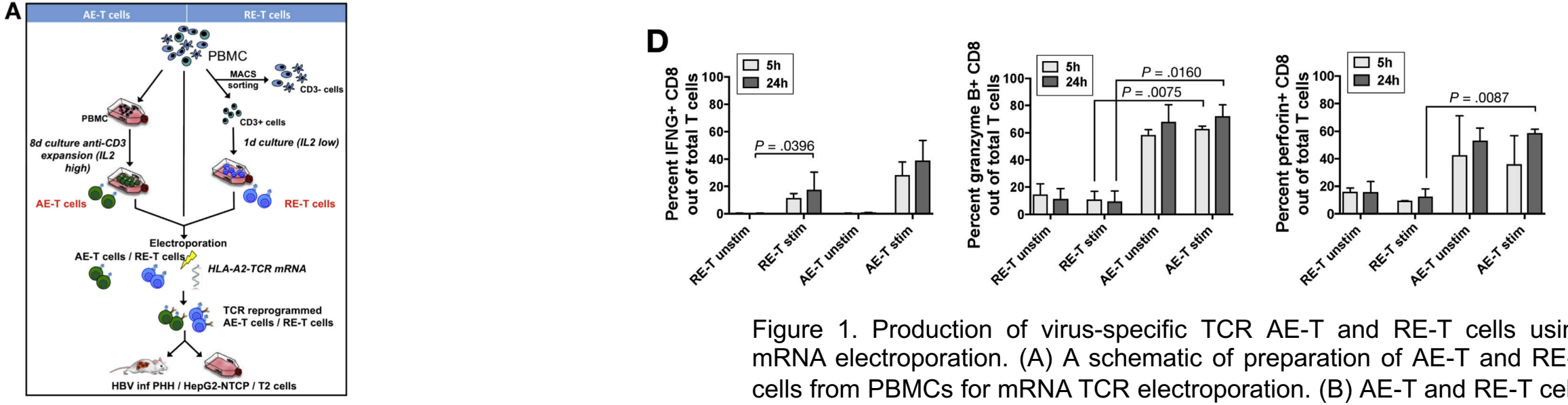


Figure 1. Production of virus-specific TCR AE-T and RE-T cells using mRNA electroporation. (A) A schematic of preparation of AE-T and RE-T cells from PBMCs for mRNA TCR electroporation. (B) AE-T and RE-T cells from healthy donors (n 1⁄4 4) were electroporated with $TCR_{HBVs183}$, TCR_{HBVc18}, or $TCR_{HCV\ NS3}$ mRNA. TCR expression was determined 24 hours after electroporation. Mock electroporated T cells served as negative control. Percentages of dextramer$^+$, TCRVβ$^+$ CD8$^+$, or CD8$^-$ cells were indicated in representative plots. (C) The mean frequencies of $TCR_{HBVs183}$ CD8$^+$ cells from 4 healthy donors were measured at indicated time points after electroporation. (D) The RE-T or AE-T cells were co-cultured with unpulsed or peptide- pulsed T2 cells for indicated times. Frequencies of IFNG, granzyme B, and perforin-producing CD8$^+$ cells out of total T cells were quantified. EP, electroporation; MACS, magnetic-activated cell sorting.

Functional Characterization of RE-T Cells

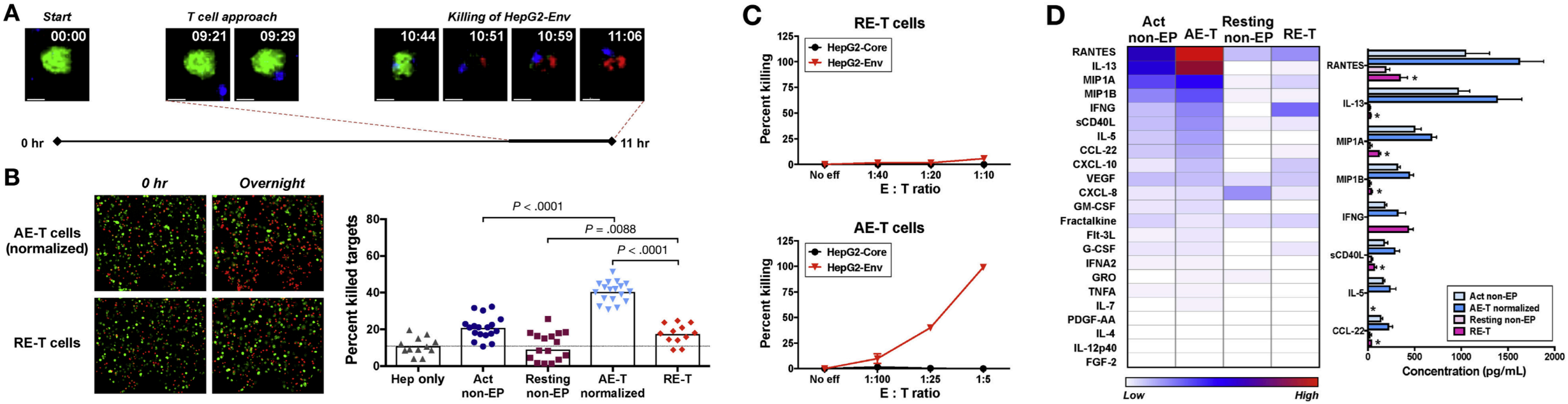


Figure 2. The RE-T cells are nonlytic and noninflammatory. (A) A representative 11-hour live-imaging timeline where $TCR_{HBVs183}$ AE-T cells were co-cultured with GFP-expressing HepG2-Env cells in a 3-D microfluidic device. The AE-T cells are displayed as blue cells, and dead cells are labeled red. The magnified maximum-intensity projections of a single HepG2-Env cell are shown at the indicated times. The 10-µm scale bars are included in each image. (B) Representative maximum intensity projections of a collagen gel region showing HepG2-Env cells (green) at 0 hours and after overnight co-culture with $TCR_{HBVs183}$ RE-T or AE-T cells. Dead cells are labeled red. The MFIs of GFP (green) and DRAQ7 (red) of each HepG2-Env cell were plotted at 0 hours and after overnight co-culture. Bar chart shows the mean percentage of killed HepG2-Env cells, and each dot represents a single experiment. Devices without T cells or co-cultured with non-electroporated T cells were included as controls. (C) Two-dimensional cytotoxicity assay performed using $TCR_{HBVs183}$ RE-T or AE-T cells and HepG2-Env or HepG2- Core cells. The mean percentages of killing obtained with different E:T ratios from 3 independent experiments are shown. (D) Heatmap of the relative mean concentration of soluble factors detected in the supernatants collected from microdevices, with indicated engineered T cells (n = 5 each). Factors with concentrations less than 10 pg/mL in all samples were removed from analysis. The bar chart shows the mean concentrations ± standard error of the mean of the top 8 secreted factors, and asterisk indicates statistically significant difference between RE-T and AE-T cells. EP, electroporation; GFP, green fluorescent protein; MFI, mean fluorescence intensity.

RE-T Cells Inhibit Virus Replication Without Hepatotoxicity

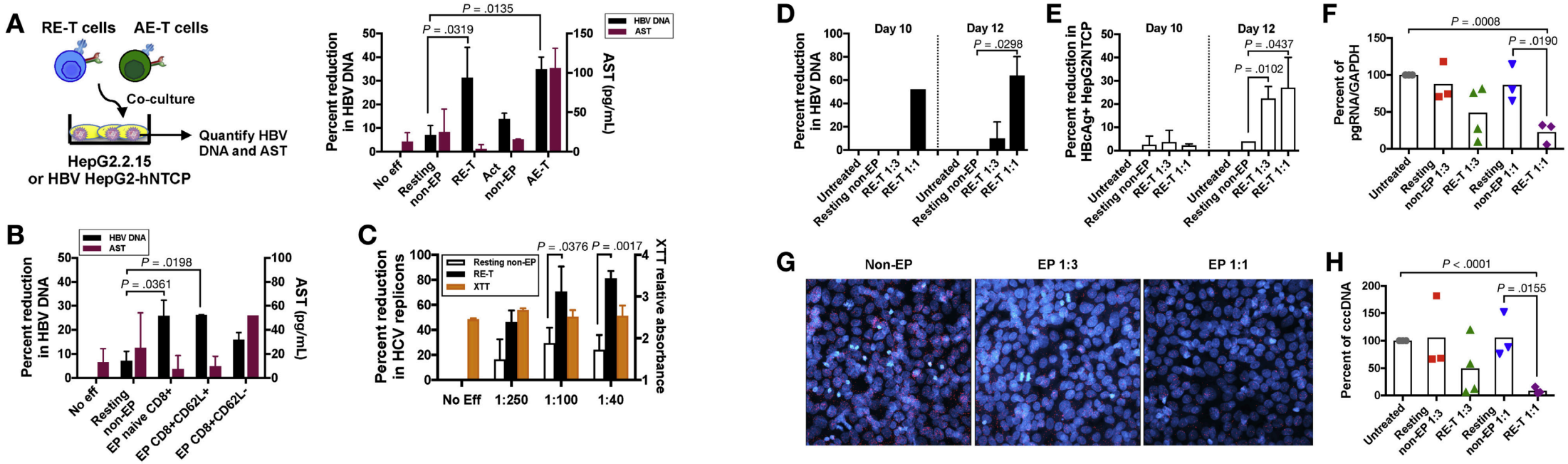


Figure 3. The RE-T cells mediate rapid antiviral effects without cytolysis. (A) Mean percentage reductions in intracellular HBV DNA ± standard deviation and means of AST ± standard deviation from 3 independent experiments of $TCR_{HBVs183}$ RE-T (n = 4) or AE-T (n = 3) cells co-cultured with HepG2.2.15 at 1:3 E:T ratio for 24 hours. (B) Naïve $CD8^+$, $CD62L^+CD8^+$ and $CD62L^-CD8^+$ memory cells were isolated from RE-T cells and co-cultured with HepG2.2.15 as in (A). Results from 2 independent experiments are shown. (C) Mean percentage reductions in HCV replication and mean XTT relative absorbance (cell viability) from 3 independent experiments of $TCR_{HCV\ NS3}$ RE-T (n = 3) or non-electroporated T cells co-cultured with $Huh7_{A2}HCV$ at indicated E:T ratios for 24 hours. (D) Mean percentage reductions in cytoplasmic HBV DNA ± standard deviation and (E) mean percentage reductions in $HBcAg^+$ HepG2-hNTCP ± standard deviation induced by $TCR_{HBVs183}$ RE-T cells co-cultured for 24 hours with HepG2-hNTCP infected for the indicated time (10 and 12 days). (F) Mean percentage HBV pgRNA normalized to GAPDH in HepG2-hNTCP at day 12 after treatment with RE-T or non-electroporated T cells. Each symbol represents a single experiment. (G) Representative confocal images of HBV pgRNA (pink dots) in HepG2-hNTCP at day 12 after treatment with RE-T or non-electroporated T cells. Nuclei were stained with 4',6-diamidino-2-phenylindole (blue). (H) Mean percentage HBV cccDNA in HepG2-hNTCP at day 12 after treatment with RE-T or non-electroporated T cells compared with untreated HepG2-hNTCP. Each dot represents a single experiment. Act, Activated; AST, aspartate amino- transferase; Eff, effect; EP, electroporated; XTT, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide.

IFNG Produced by RE-T Cells Does Not Inhibit HBV Replication

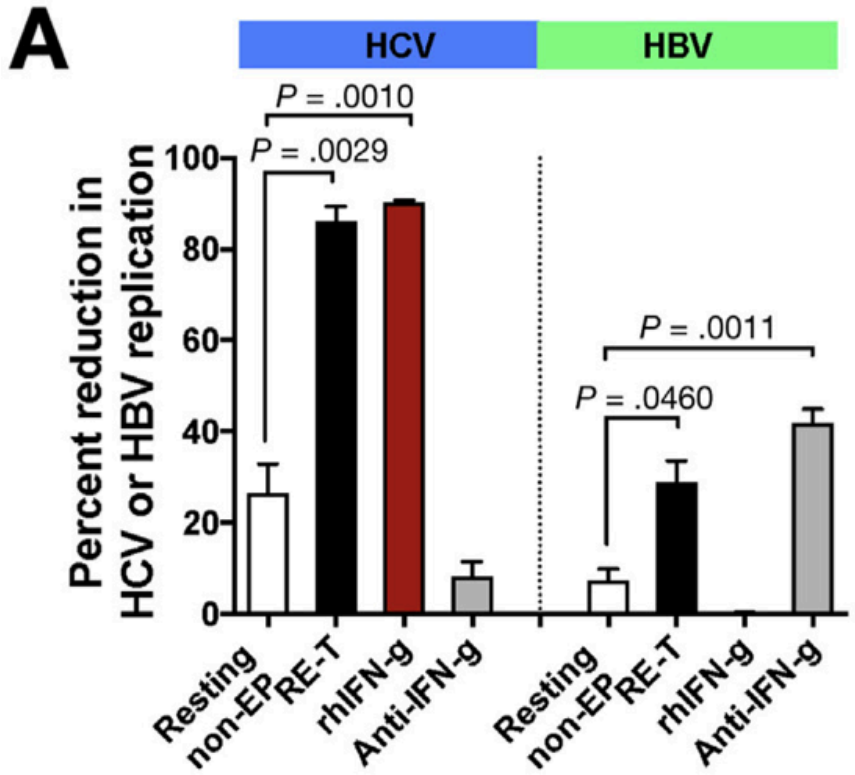


Figure 4. Increased expression of LTBR ligands on RE-T cells after antigen-specific TCR recognition activates intracellular APOBEC3. (A) Mean percentage reductions in HCV or HBV replication ± standard deviation of 2 independent experiments of $Huh7_{A2}$HCV and HepG2.2.15 treated with TCR–RE-T or non-electroporated T cells at a 1:3 E:T ratio in the absence or presence of 10 µg/mL anti-IFNG antibodies or 200 IU/mL rhIFNG alone for 24 hours.

RE-T Cells Activate APOBEC3 Antiviral Pathway for Rapid HBV Inhibition

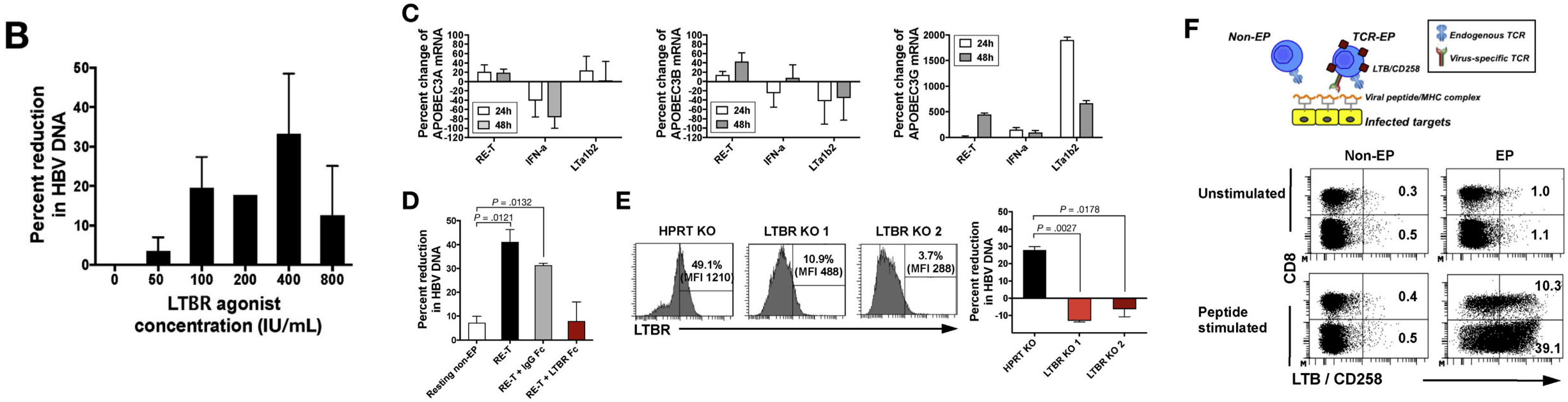


Figure 4. Increased expression of LTBR ligands on RE-T cells after antigen-specific TCR recognition activates intracellular APOBEC3. (B) Mean percentage reductions in intracellular HBV DNA ± standard deviation of 2 independent experiments of HepG2.2.15 treated with indicated doses of LTBR agonist for 48 hours. (C) Percent change of A3A, A3B, and A3G mRNA ± standard deviation of triplicates in HepG2.2.15 treated with RE-T or non-electroporated T cells at a 1:3 E:T ratio or 1000 IU/mL IFN-alfa or 400 IU/mL LTBR agonist for the indicated times compared with treatment with non-electroporated T cells. (D) Mean percent reductions in intracellular HBV DNA ± standard deviation of 2 independent experiments of HepG2.2.15 treated with RE-T or non-electroporated T cells at a 1:3 E:T ratio for 24 hours in the absence and presence of soluble LTBR Fc chimera antibody or a control IgG Fc antibody. (E) Frequency of cells expressing LTBR and the MFI of LTBR expression in LTBR-knockout (clones 1 and 2) and HPRT-knockout HepG2.2.15. Bar chart shows mean percent reductions in intracellular HBV DNA ± standard deviation of 3 independent experiments of knockout clones co-cultured with $TCR_{HBVs183}$ RE-T cells at a 1:3 E:T ratio for 24 hours. (F) RE-T or non-electroporated T cells were co-cultured with unpulsed or peptide-pulsed T2 cells for 24 hours, and the frequencies of RE-T cells expressing LTB and CD258 from one representative experiment are shown.

RE-T Cells Transiently Express LTBR Ligands

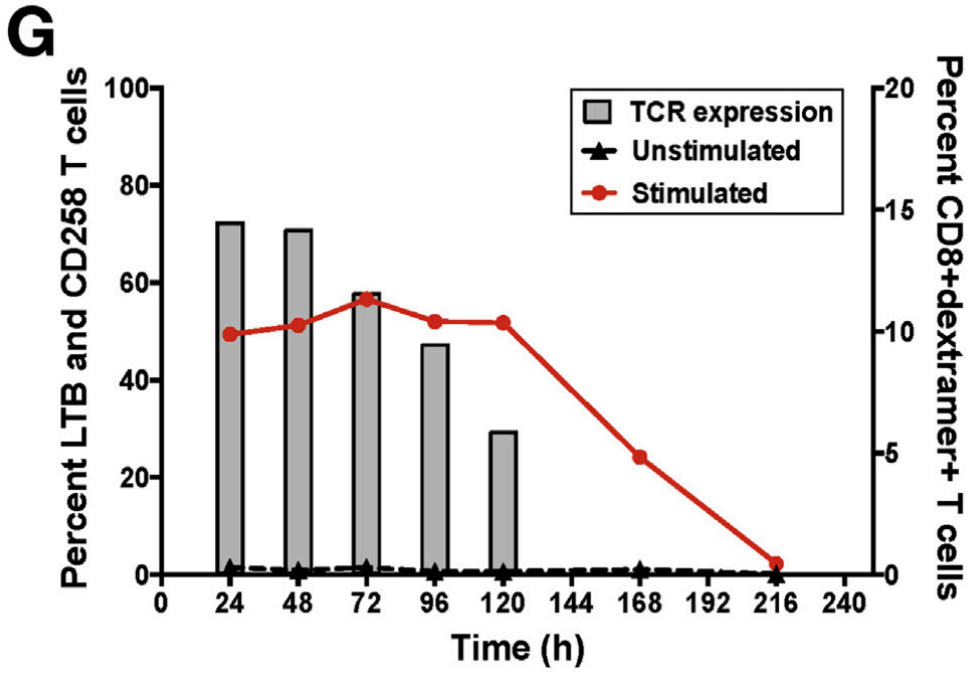


Figure 4. Increased expression of LTBR ligands on RE-T cells after antigen-specific TCR recognition activates intracellular APOBEC3. (G) The frequencies of $CD8^{+}$dextramer^{+} T cells (bars) and LTB- and CD258-expressing T cells out of total lymphocytes in unstimulated (dotted black line) and peptide- stimulated (red line) RE-T cells were determined longitudinally at indicated times. EP, electroporated; KO, knockout; MFI, mean fluorescent intensity.

RE-T Cells Activate LTB and APOBEC3B in HBV-Infected Primary Human Hepatocytes Without Inducing Liver Inflammation

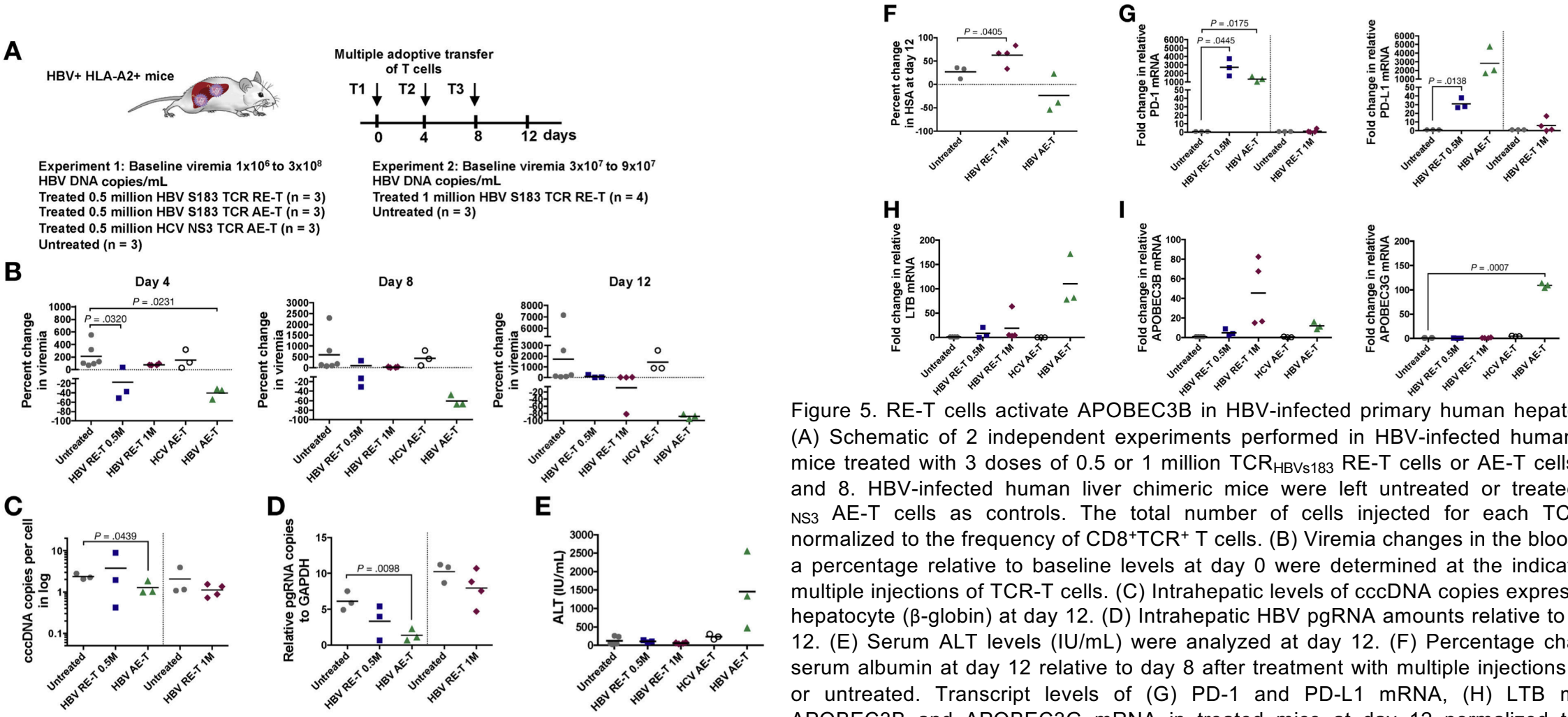


Figure 5. RE-T cells activate APOBEC3B in HBV-infected primary human hepatocytes in mice. (A) Schematic of 2 independent experiments performed in HBV-infected human liver chimeric mice treated with 3 doses of 0.5 or 1 million $TCR_{HBVs183}$ RE-T cells or AE-T cells on days 0, 4, and 8. HBV-infected human liver chimeric mice were left untreated or treated with $TCR_{HCV\ NS3}$ AE-T cells as controls. The total number of cells injected for each TCR-T cells was normalized to the frequency of $CD8^+TCR^+$ T cells. (B) Viremia changes in the blood expressed as a percentage relative to baseline levels at day 0 were determined at the indicated times upon multiple injections of TCR-T cells. (C) Intrahepatic levels of cccDNA copies expressed per human hepatocyte (β-globin) at day 12. (D) Intrahepatic HBV pgRNA amounts relative to GAPDH at day 12. (E) Serum ALT levels (IU/mL) were analyzed at day 12. (F) Percentage change in human serum albumin at day 12 relative to day 8 after treatment with multiple injections of TCR-T cells or untreated. Transcript levels of (G) PD-1 and PD-L1 mRNA, (H) LTB mRNA, and (I) APOBEC3B and APOBEC3G mRNA in treated mice at day 12 normalized to GAPDH and ribosomal protein L30 and expressed as fold change compared with untreated mice. ALT, alanine aminotransferase; M, mol/L.