The Journal of Clinical Investigation

RESEARCH ARTICLE

Lymphocytes transiently expressing virus-specific T cell receptors reduce hepatitis B virus infection

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Submitted: January 24, 2017; **Accepted:** June 1, 2017.
Reference information: *J Clin Invest*. 2017;127(8):3177–3188.
https://doi.org/10.1172/JCI93024.

Abstract

Adoptive transfer of T cells engineered to express a hepatitis B virus–specific (HBV-specific) T cell receptor (TCR) may supplement HBV-specific immune responses in chronic HBV patients and facilitate HBV control. However, the risk of triggering unrestrained proliferation of permanently engineered T cells raises safety concerns that have hampered testing of this approach in patients. The aim of the present study was to generate T cells that transiently express HBV-specific TCRs using mRNA electroporation and to assess their antiviral and pathogenetic activity in vitro and in HBV-infected human liver chimeric mice. We assessed virological and gene-expression changes using quantitative reverse-transcriptase PCR (qRT-PCR), immunofluorescence, and Luminex technology. HBV-specific T cells lysed HBV-producing hepatoma cells in vitro. In vivo, 3 injections of HBV-specific T cells caused progressive viremia reduction within 12 days of treatment in animals reconstituted with haplotype-matched hepatocytes, whereas viremia remained stable in mice receiving irrelevant T cells redirected toward hepatitis C virus–specific TCRs. Notably, increases in alanine aminotransferase levels, apoptotic markers, and human inflammatory cytokines returned to pretreatment levels within 9 days after the last injection. T cell transfer did not trigger inflammation in uninfected mice. These data support the feasibility of using mRNA electroporation to engineer HBV TCR–redirected T cells in patients with chronic HBV infection.

mRNA TCR–expressing T cells display antiviral efficacy in vitro.

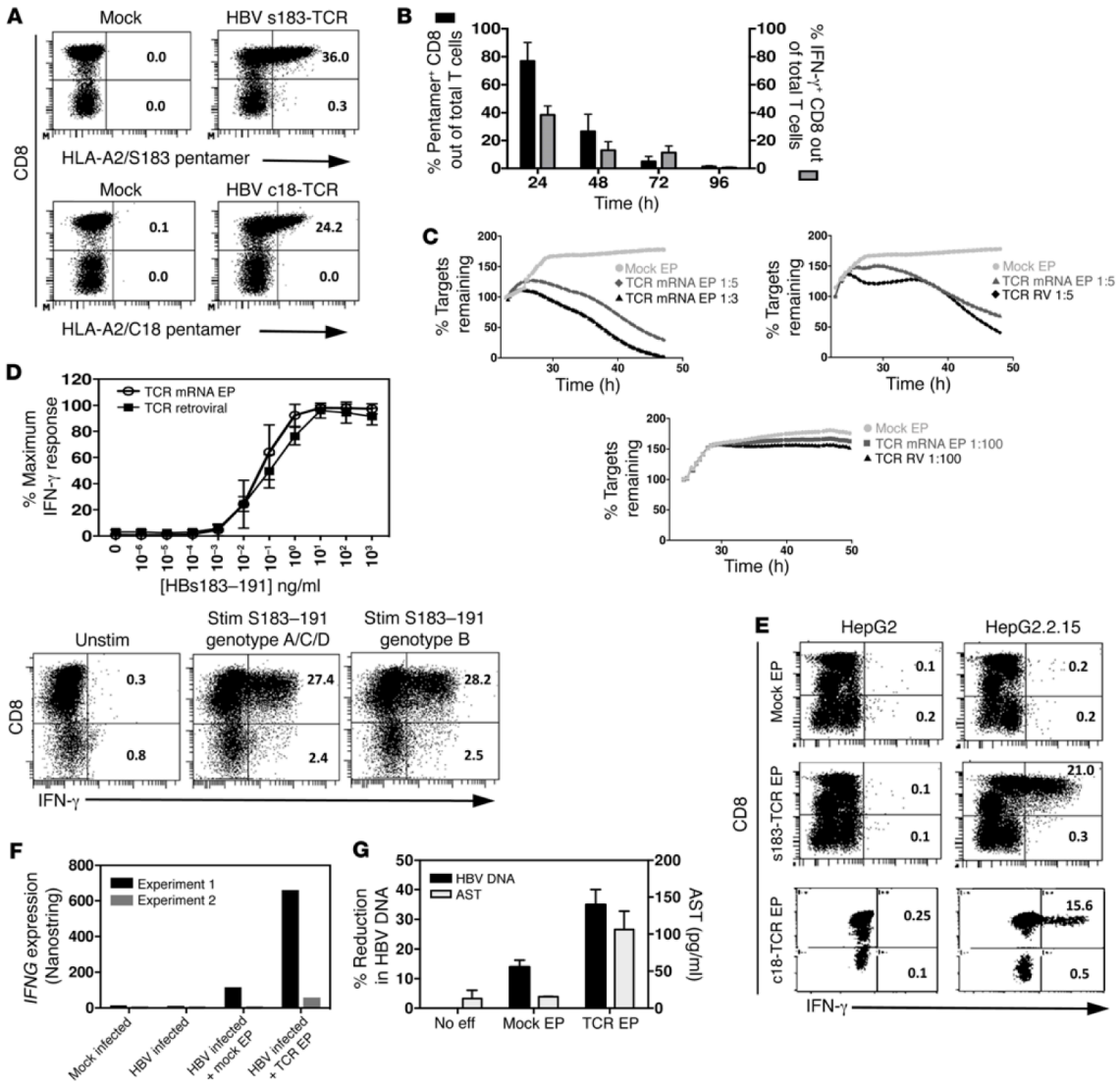

Figure 1. Lytic and antiviral function of mRNA HBV–specific TCR–electroporated T cells in vitro. (**A**) Activated T cells were electroporated with HBV s183–TCR or c18-TCR mRNA, and TCR expression was determined 24 hours after electroporation. Mock electroporated T cells served as negative control. Shown are representative plots. The percentages of HLA-A2/pentamer+ cells out of CD8+ or CD8− T cells are indicated. (**B**) TCR expression on electroporated cells was measured longitudinally from 24 hours to 96 hours. Electroporated T cells were cocultured with their respective peptide-pulsed T2 cells for 18 hours, and the frequencies of IFN-γ–producing CD8+ T cells out of total lymphocytes were quantified. (**C**) The ability of mRNA TCR–electroporated T cells to lyse HepG2.2.15 HBV–producing cells at 1:3, 1:5, and 1:100 E:T ratios within 24 hours after T cell addition was compared with that of retroviral transduced (TCR RV) T cells. (**D**) Sensitivity of T cell activation, displayed as percentage of maximum IFN-γ response using mRNA TCR–electroporated T cells compared with retroviral-transduced T cells (upper panel). MRNA HBV s183–TCR–electroporated T cells were cocultured with HBV s183–191 genotype B (FLLTKILTI) or genotype A/C/D (FLLTRILTI) peptide-loaded T2 cells. The percentages of CD8+ or CD8− T cells producing IFN-γ are indicated (lower panel). (**E**) Mock, mRNA HBV s183–TCR, or c18-TCR–electroporated T cells were cocultured with either HepG2 or HepG2.2.15 cells for 24 hours. The percentages of CD8+ or CD8− T cells producing IFN-γ are indicated. (**F**) mRNA HBV s183–TCR or c18-TCR–electroporated T cells were cocultured with mock or HBV-infected HepG2-NTCP for 24 hours, and *IFNG* gene expression was determined using NanoString analysis. (**G**) Mock or mRNA HBV s183–TCR–electroporated T cells were cocultured with HepG2.2.15 cells at a 1:3 E:T ratio for 24 hours, and intracellular HBV DNA was quantified by real-time quantitative PCR (qPCR). AST levels were determined in coculture media. Shown are means of percentage reduction in intracellular HBV DNA ± SD (black bars) and means of AST ± SD (gray bars) from 3 independent experiments (right panel).

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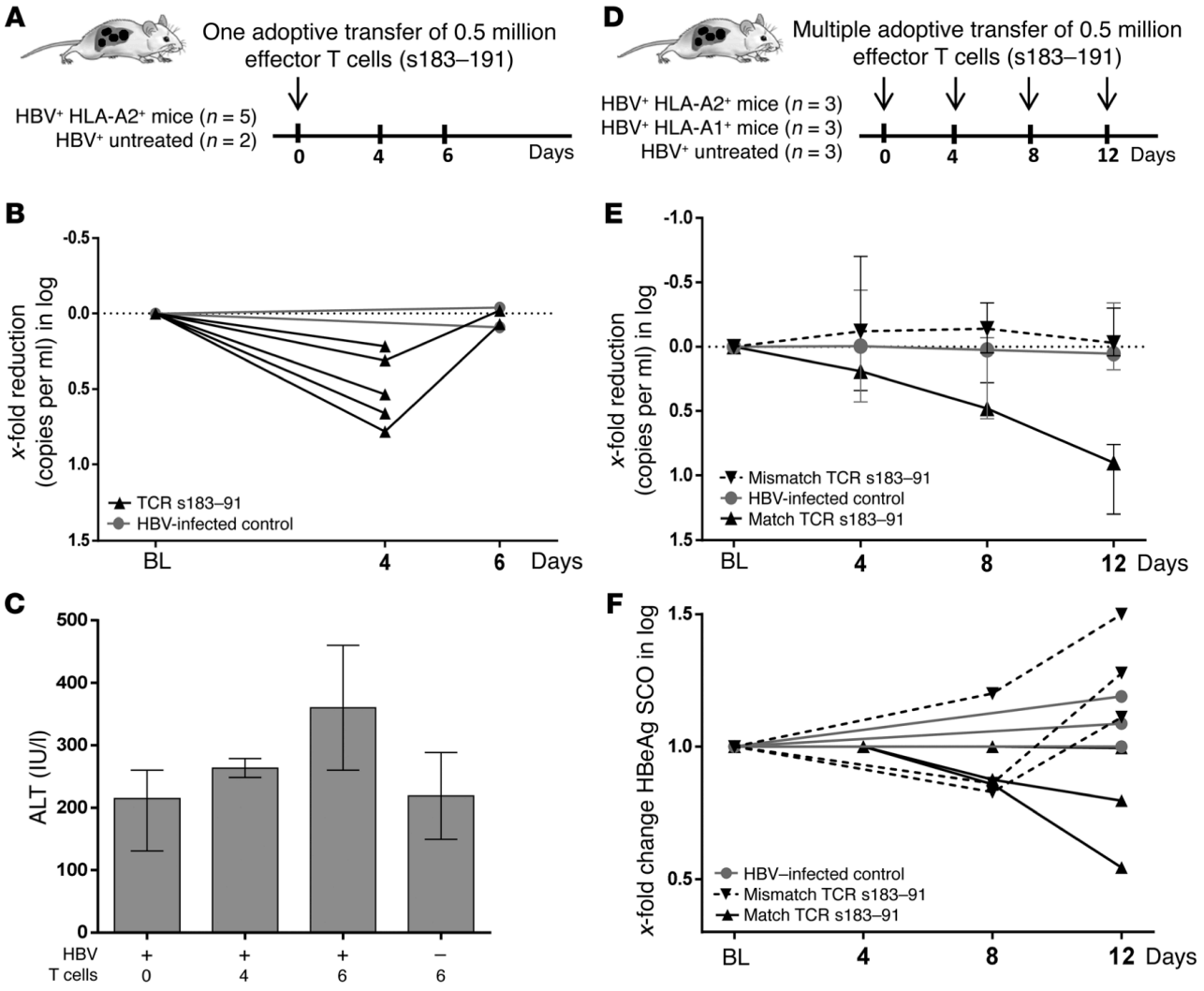

Figure 2. mRNA HBV–specific TCR–electroporated T cells show antiviral efficacy in vivo. (**A**) Schematic representation of the experiment performed to assess the effect of 1 single injection of electroporated effector T cells in high viremic mice reconstituted with haplotype-matched hepatocytes. (**B**) Viremia changes relative to baseline levels determined after 4 and 6 days in individual mice upon 1 injection of mRNA HBV s183–TCR T cells (*n* = 5) and in untreated controls (*n* = 2). (**C**) ALT levels determined in HBV-infected and uninfected mice receiving a single injection of effector T cells. (**D**) Schematic representation of the experiment performed to assess the antiviral effect of multiple injections of electroporated effector T cells in high viremic mice reconstituted either with haplotype-matched (*n* = 3) or -mismatched (*n* = 3) human hepatocytes and in comparison with mice that were left untreated (*n* = 3). (**E**) Median viremia changes determined within each group depicted in **D** and relative to baseline levels determined in individual mice upon 3 injections of mRNA HBV s183–TCR T cells. Blood was taken 4, 8, and 12 days after the first T cell injection. (**F**) Median changes in levels of circulating HBeAg were determined by ELISA in all animal groups. BL, baseline.

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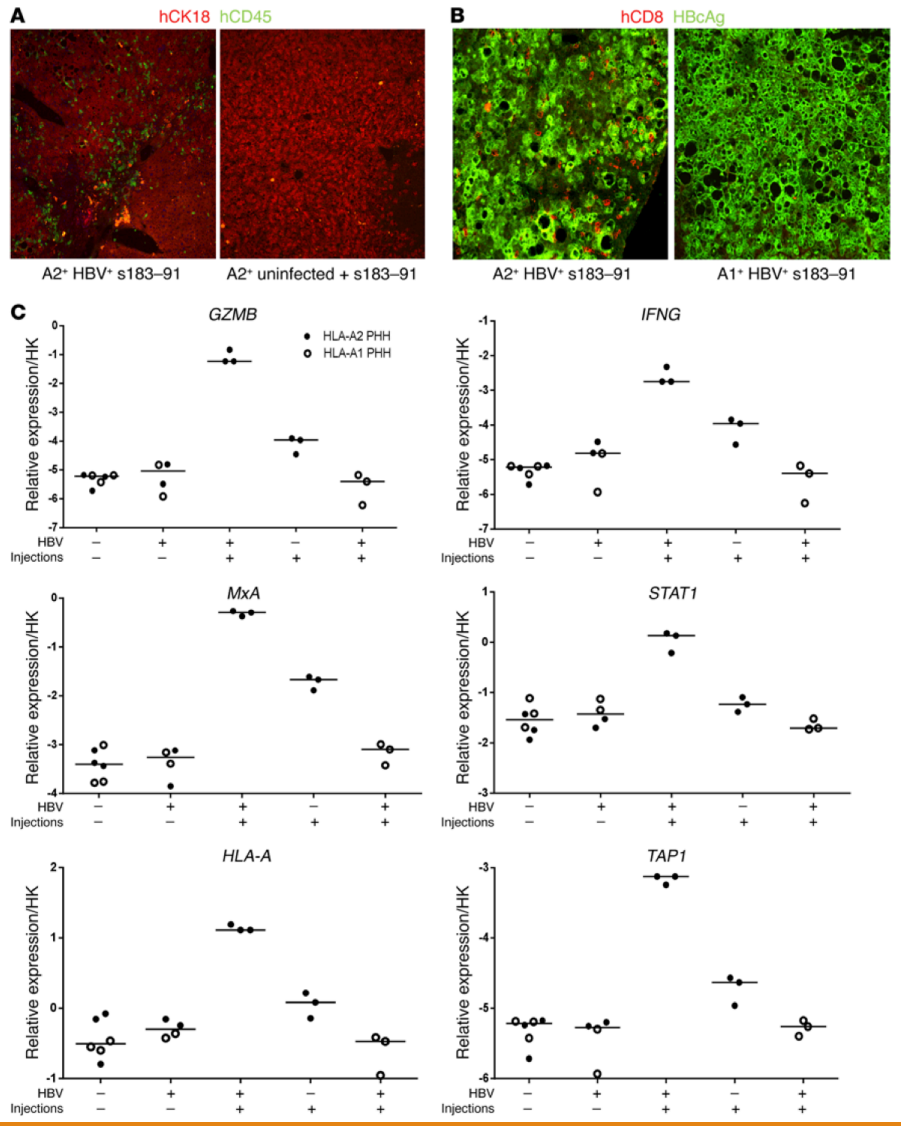

Figure 3. mRNA HBV–specific TCR–electroporated T cells are specifically recruited and activated in livers of haplotype-matched HBV-infected mice. (**A**) Liver tissues of humanized HBV-infected mice and uninfected mice that underwent 3 injections of HBV s183–TCR T cells and were sacrificed 4 days after the third T cell transfer were used for immunofluorescence. Human hepatocytes were identified using human-specific CK18 Abs (red). Transferred human immune cells were visualized using human-specific CD45 Abs (green). (**B**) Liver tissues of HBV-infected mice adoptively transferred with either HLA-A2– or HLA-A1–presenting human hepatocytes were costained with HBV core–specific Ab (green) and human CD45-specific Ab (red). (**C**) Transcript levels of human T cell response–related genes (*GZMB* and *IFNG*) and human ISGs (*MxA*, *STAT1*, *TAP1*, and *HLA-A*) were measured by quantitative reverse-transcrip- tase PCR (qRT-PCR) and normalized against human housekeeping transcripts. Statistical analysis was performed with GraphPad Prism 6 software.

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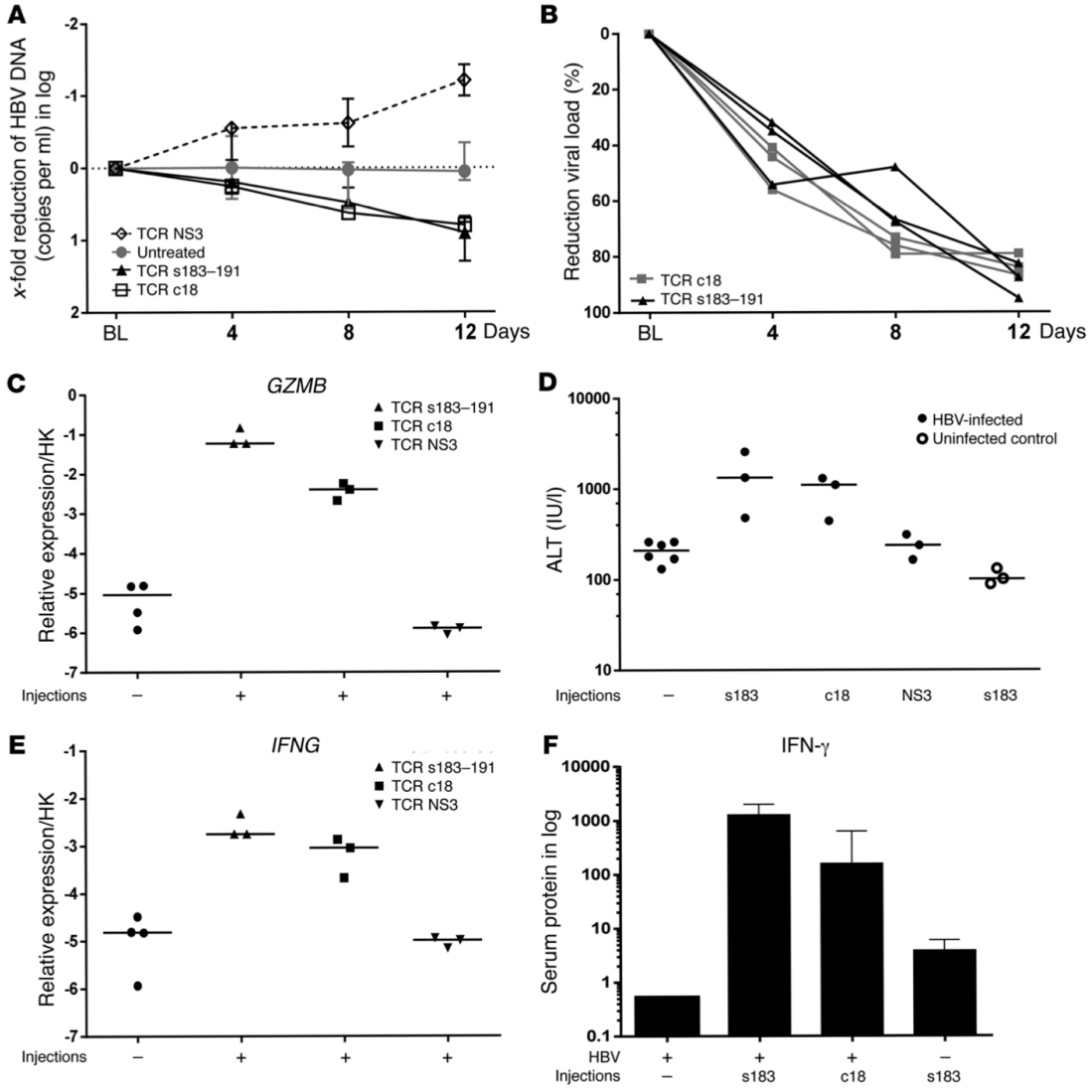

Figure 4. Antiviral and inflammatory events after in vivo multiple injections of different mRNA HBV–specific TCR–electroporated T cells. (**A**) Median viremia changes relative to baseline levels were determined as indicated after 4, 8, and 12 days upon multiple injections of mRNA HBV s183–TCR T cells (*n* = 3), mRNA c18-TCR T cells (*n* = 3), and mRNA mock TCR T cells (*n* = 3) as well as in untreated controls (*n* = 6). (**B**) Individual reduction (shown as per- centages) of viremia relative to baseline levels determined on days 4, 8, and 12 upon transfer of mRNA s183–191 T cells and mRNA c18 T cells. Transcrip- tional changes of human T cell response–related genes (**C**, *GZMB*; **E**, *IFNG*) were measured by qRT-PCR and normalized against human housekeeping transcripts. (**D**) ALT levels were determined in uninfected (*n* = 3) and HBV-infected mice receiving multiple injections of effector T cells presenting s183 (*n* = 3), c18 (*n* = 3), or HCV NS3 as mock control (*n* = 3) in comparison with HBV-infected control mice (*n* = 6). (**F**) Median changes in human IFN-γ serum pro- tein levels were determined by multiplex measurement in HBV-infected (s183 median = 1280 ng/ml or c18 median = 160 ng/ml) as well as uninfected (s183 median = 3.6 ng/ml) mice that received 3 injections of HBV-specific effector T cells relative to HBV-infected control mice (*n* = 4; median = 0.53 ng/ml).

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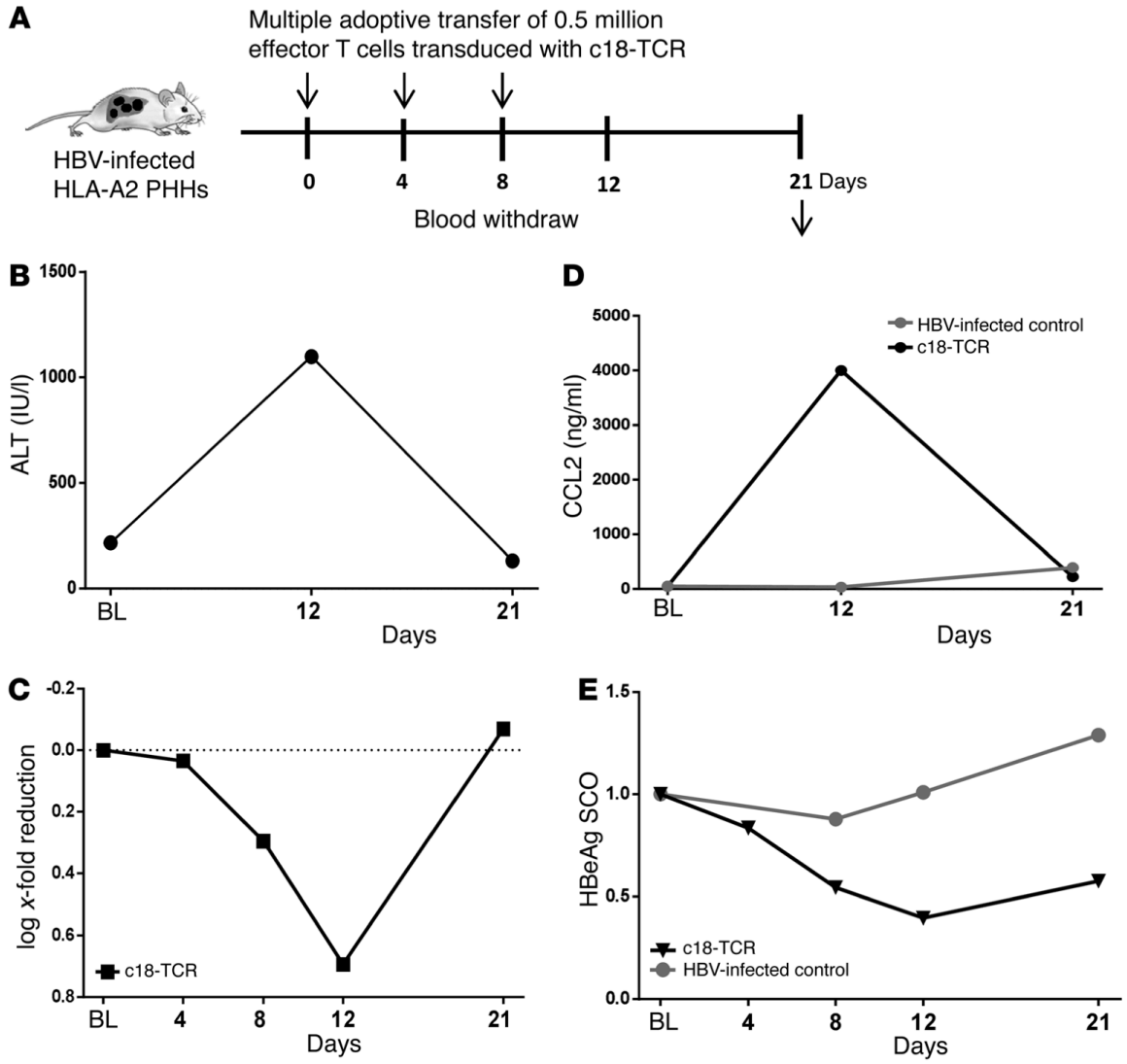

Figure 5. Adoptive transfer of mRNA HBV–specific TCR–electroporated T cells leads to temporary limited liver inflammation and cell damage. (**A**) Schematic representation of the experiment performed to assess the effect of multiple injections of electroporated effector T cells after treatment cessation both on inflammatory and virological parameters. (**B**) ALT levels were determined in 1 HBV-infected animal shortly before T cell injection (baseline) after receiving 3 injections of HBV-specific c18-TCR T cells (day 12) and 9 days after the last T cell injection (day 21). (**C**) Longitudinal changes in viremia relative to baseline were determined at 4, 8, 12, and 21 days after the first T cell trans- fer as depicted in **A**. (**D**) Serum protein levels of CCL2 were determined in both 1 HBV-infected control mouse (gray) and 1 mouse receiving multiple T cell injections (c18-TCR), and that was monitored for 21 days. (**E**) Longitudinal changes in levels of circulating HBeAg were determined by ELISA in the same mice described in **D**.