Research Article

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Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines

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Abstract

Background & Aims: Virus-specific T cells capable of controlling HBV and eliminating hepatocellular carcinoma (HCC) expressing HBV antigens are deleted or dysfunctional in patients with chronic HBV or HBV-related HCC. The goal of this study was to determine if T cell receptor (TCR) gene transfer can reconstitute HBV-specific T cell immunity in lymphocytes of chronic HBV patients and investigate whether HCC cells with natural HBV–DNA integration can be recognized by genetically modified T cells.

Methods: We used vector-mediated gene transfer to introduce HLA-A2-restricted, HBV-specific TCRs into T cells of chronic HBV as well as HBV-related HCC patients.

Results: The introduced TCRs were expressed on the cell surface, evidenced by Vb and pentamer staining. TCR transduced T cells produced IFN-c, TNF-a, IL-2, and lysed HBV infected hepato cyte-like cell lines. Furthermore, HCC cell lines with natural HBV–DNA integration could be recognized by HBV-specific TCR- re-directed T cells.

Conclusions: TCR re-directed HBV-specific T cells generated from PBMC of chronic HBV and HBV-related HCC patients were multi- functional and capable of recognizing HBV-infected cells and HCC tumor cells expressing viral antigens from naturally integrated HBV DNA. These genetically modified T cells could be used to reconstitute virus-specific T cell immunity in chronic HBV patients and target tumors in HBV-related HCC.

Expression of introduced TCR in chronic HBV patients

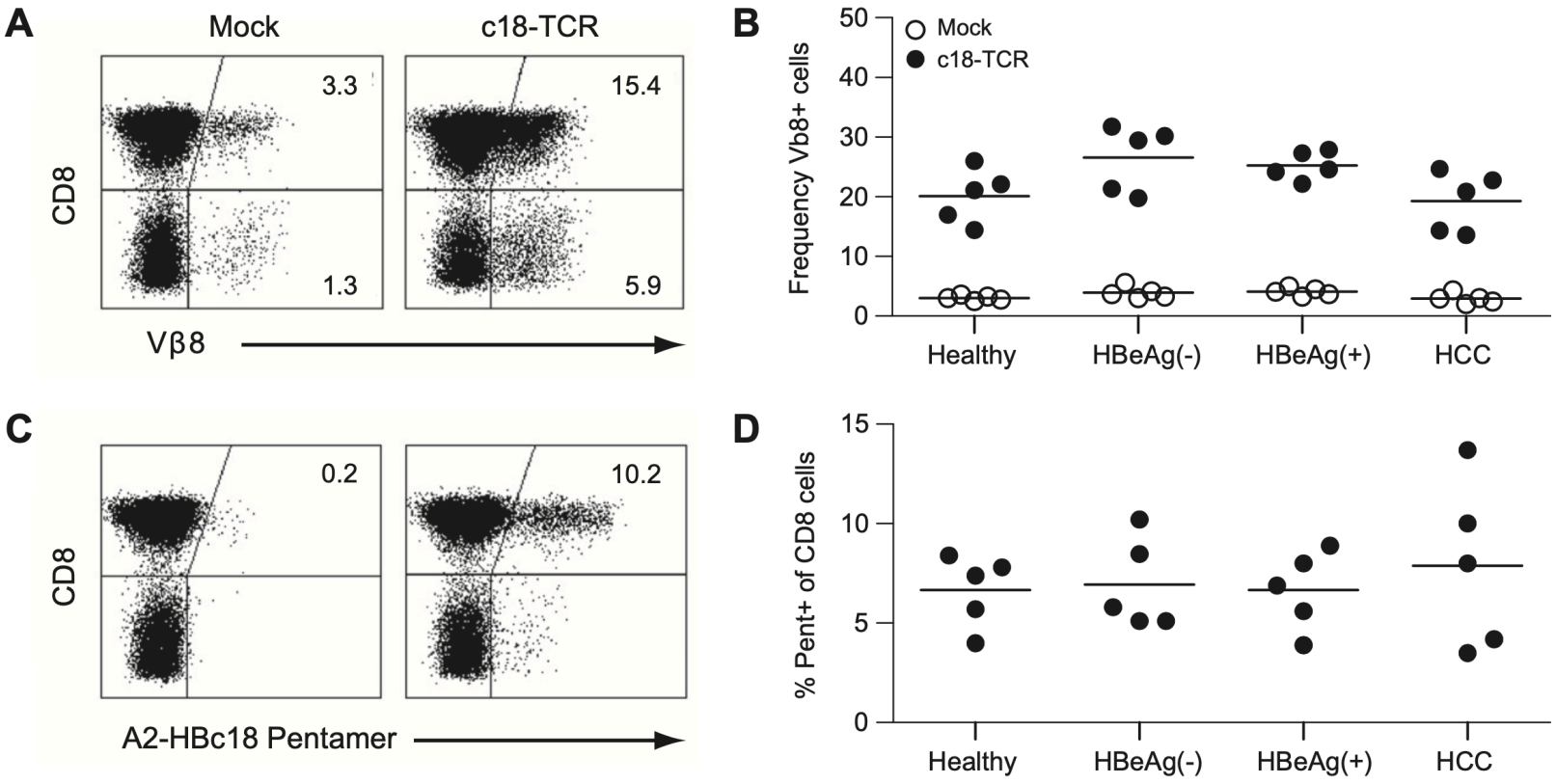

Fig. 1. Expression of introduced TCR. (A) Dot plot of Vb8.2 expression in mock or c18-TCR transduced T cells from a representative HBeAg- patient. (B) Mean frequency of CD8+ Vb8.2+ T cells in mock and c18-TCR transduced T cells from five patients in each group. (C) Dot plot of HLA-A2-HBc18-27 pentamer staining in mock or c18-TCR transduced T cells from a representative HBeAg- patient. (D) Mean CD8+ pentamer+ T cells, in mock and c18-TCR transduced T cells from five patients in each group. There was no statistically significant difference between pentamer+ or Vb8+ cells from each patient group using one-way ANOVA analysis ($p > 0.05$).

TCR gene-modified T cells are multi-functional

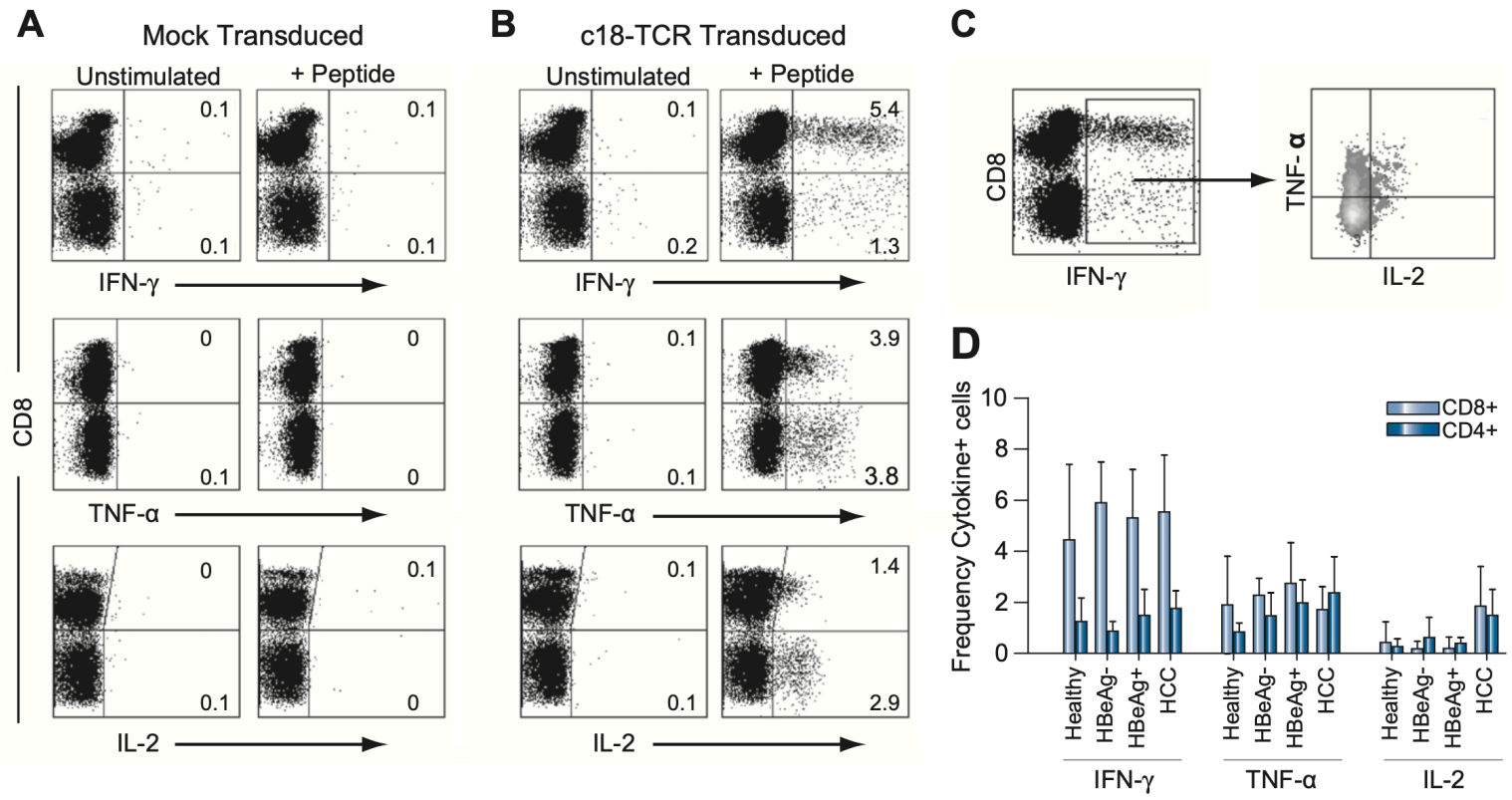

Fig. 2. Functional profile of TCR transduced T cells. Dot plots from a representative HBeAg- (A) mock and (B) c18-TCR transduced T cells, +/- peptide stimulation, stained for CD8 and IFN-γ (top row), TNF-α (middle row) and IL-2 (bottom row). (C) TNF-α and IL-2 production by IFN-γ+ cells to demonstrate multi-functionality of TCR transduced cells. (D) Mean frequency of cytokine positive cells from all patients in each group. Data shown is mean ± standard deviation of the frequency of cytokine positive cells from each patient group.

Sensitivity of TCR transduced T cells

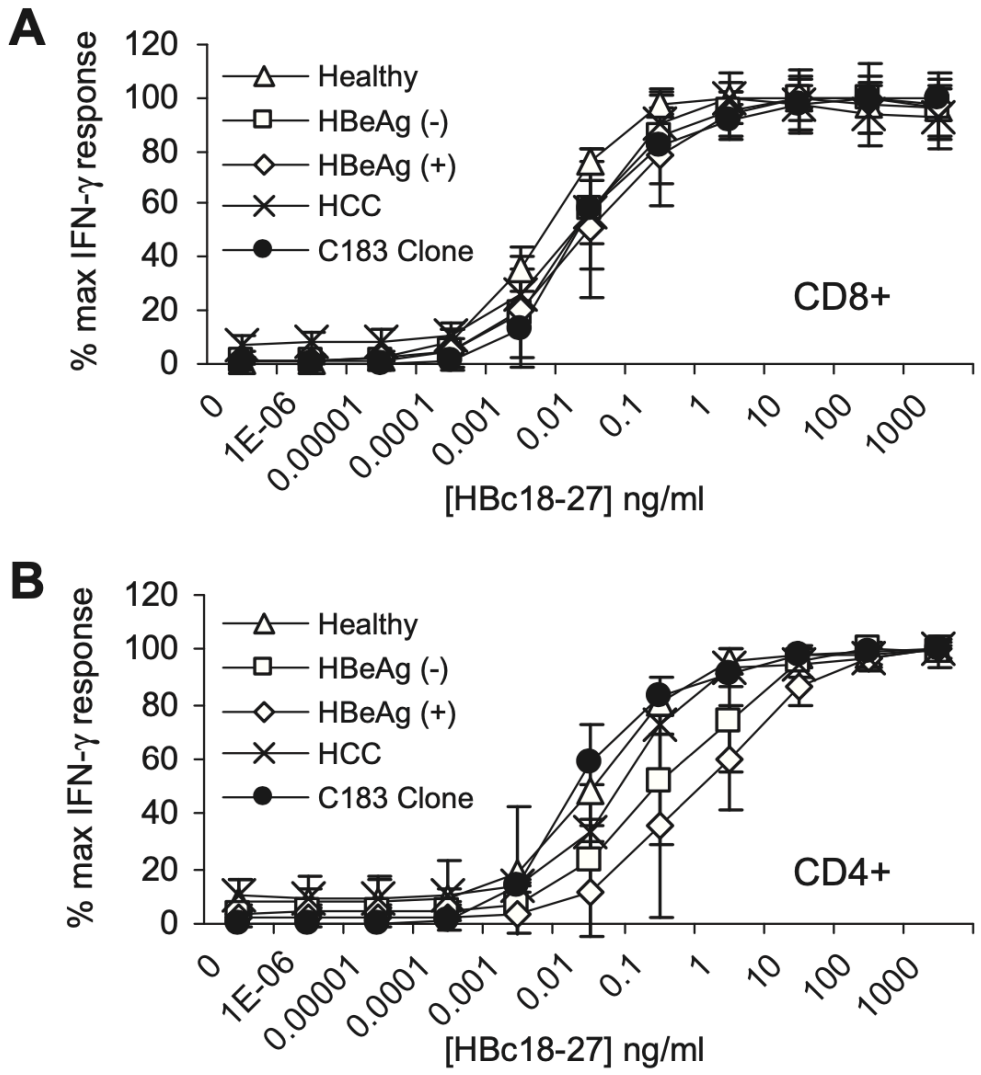

Fig. 3. Sensitivity of TCR transduced T cell activation in (A) CD8 or (B) CD4 c18-TCR transduced T cells from each patient group compared to the original T cell clone, C183. Results are displayed as mean of five patients from each group, +/- standard deviation of percent maximum IFN-γ response obtained from intracellular cytokine staining.

Cytotoxicity of TCR transduced T cells

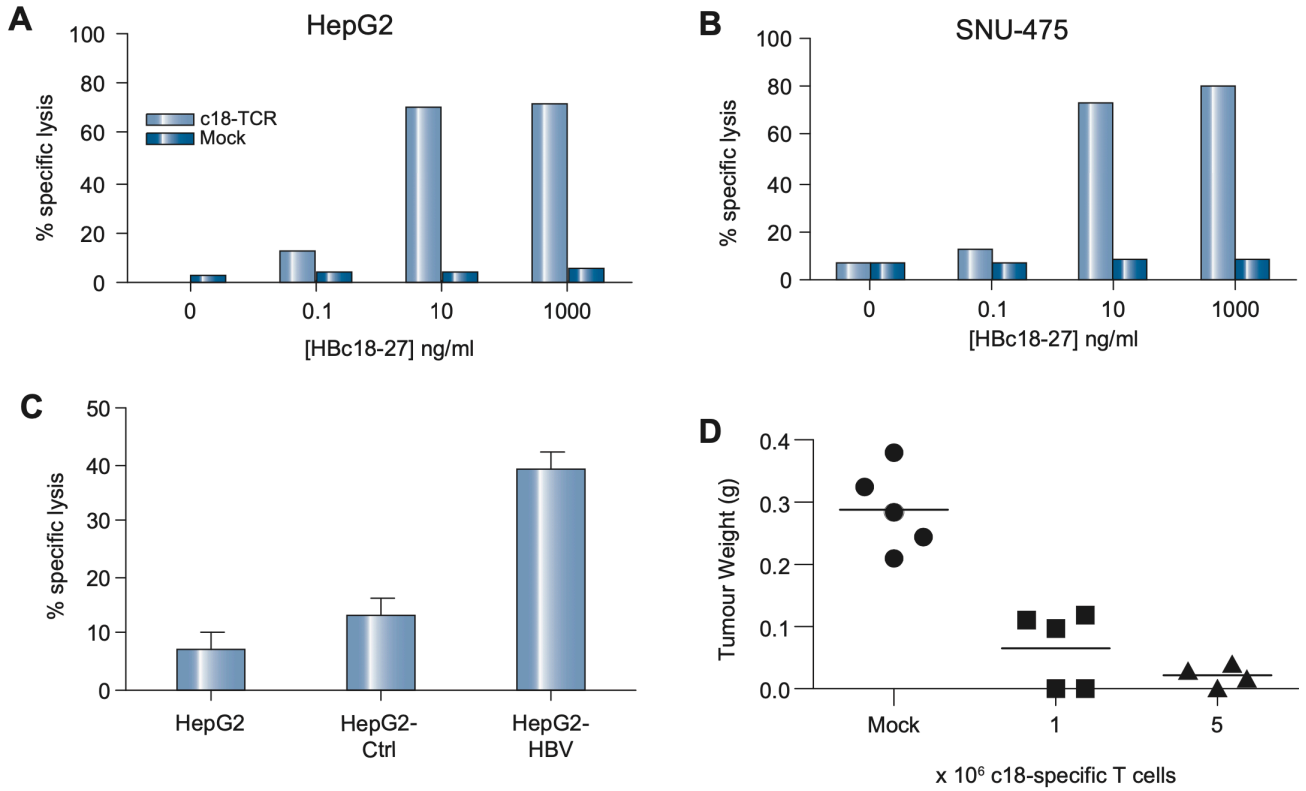

Fig. 4. Lysis of hepatocyte cell lines. Dose dependent lysis of peptide loaded (A) HepG2 and (B) SNU-475 HCC by mock or c18-TCR transduced T cells. (C) Lysis of HBV expressing HepG2 cells (HepG2-HBV) by c18-TCR transduced T cells. HepG2 cells or HepG2 with empty expression cassette (HepG2-ctrl) served as negative control. Each panel is representative of at least three separate experiments with T cells from different patients. (D) In vivo cytotoxicity assay. Size of subcutaneous tumors 15 d after adoptive transfer of 1 or 5 x 10^6 c18-TCR T cells.

Recognition of HCC lines naturally expressing HBV antigen

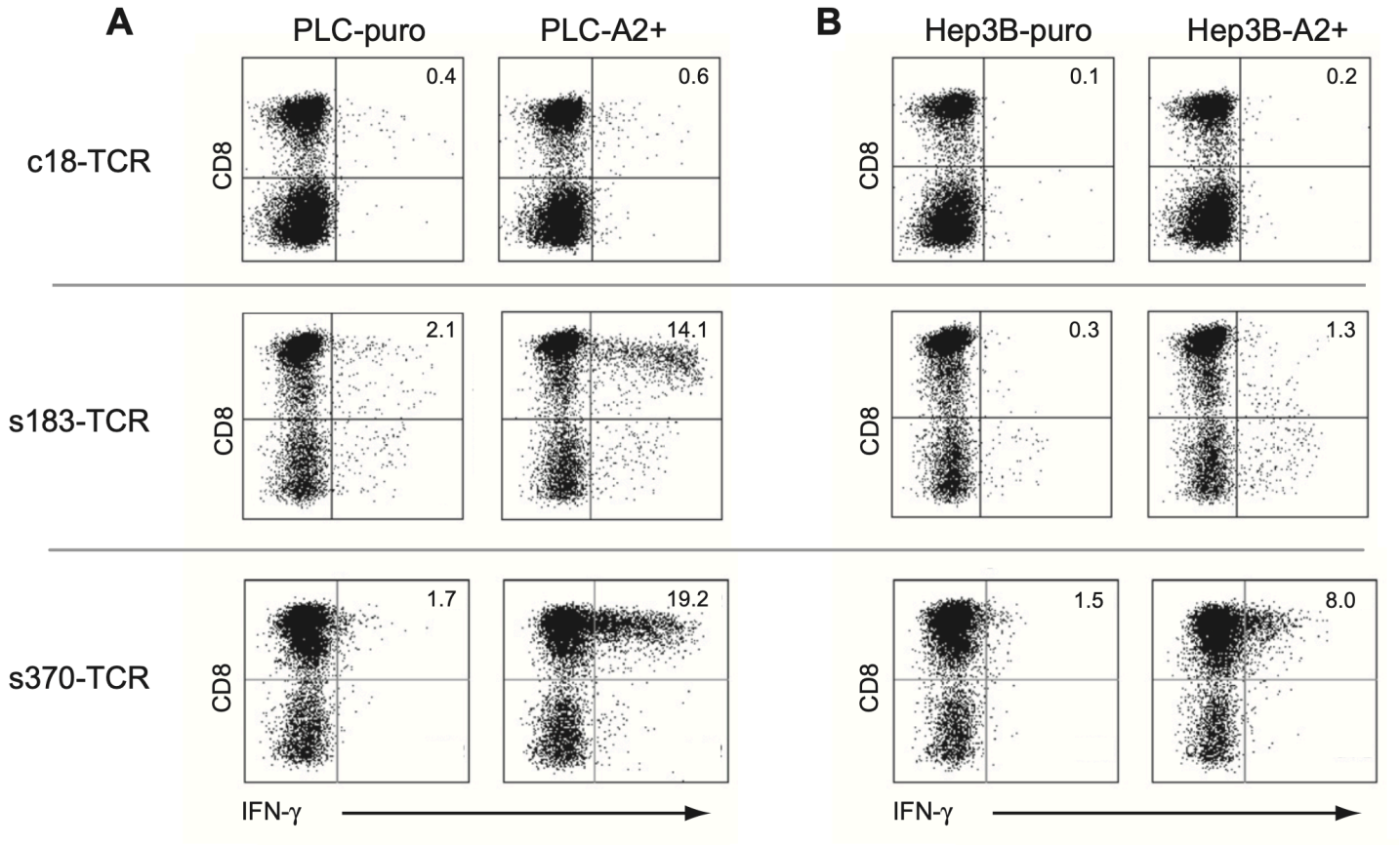

Fig. 5. Recognition of HCC cell lines naturally expressing HBV proteins. C18-TCR (top row), s183-TCR (middle row), and s370-TCR (bottom row) transduced T cell IFN-c production following overnight co-culture with (A) HLA-A2 negative PLC-puro and HLA-A2+ PLC-A2 or (B) Hep3B-puro and Hep3B-A2 cells. T cells used for this experiment were derived from healthy donors.

Recognition of HCC lines naturally expressing HBV antigen

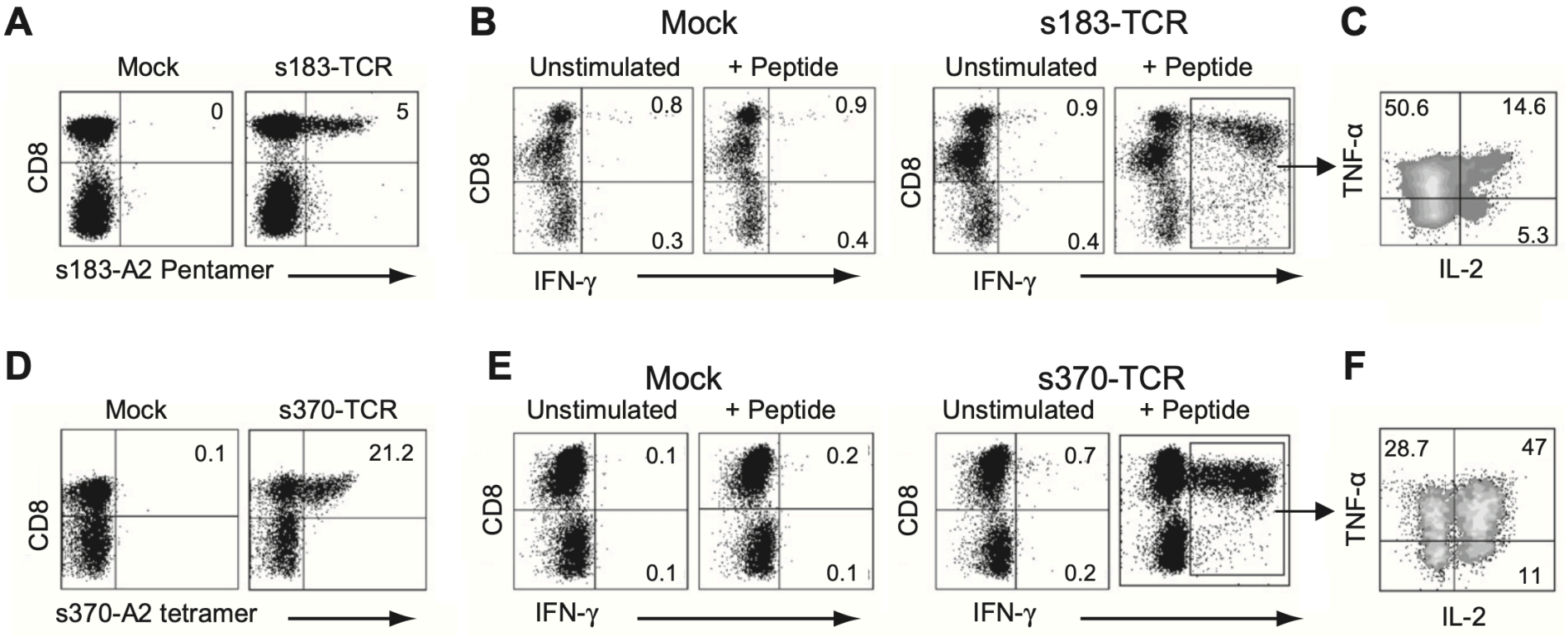

Fig. 6. Characterization of s183- and s370-TCR expression and transduced T cell function. HLA-A2 pentamer staining of mock and (A) s183-TCR or (D) s370-TCR transduced T cells. IFN-γ production in mock and (B) s183-TCR or (E) s370-TCR transduced T cells. Transduction with (C) s183-TCR and (F) s370-TCR generates multi-functional T cells. Panel shows TNF-α and IL-2 production by IFN-γ+ T cells.