Fc-fused interleukin-7 (NT-I7) promotes expansion and mobilization of virus-specific PD-1⁺TCF1⁺ CD8 T cells during chronic viral infection

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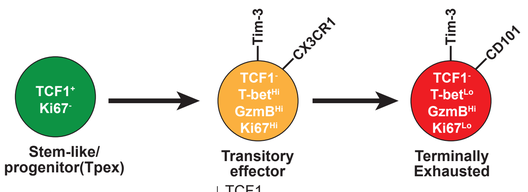
Abstract

PD-1⁺TCF1⁺ stem-like or progenitor exhausted CD8 T cells are a distinct subset identified as a crucial resource for sustained immune responses in chronic viral infections and cancer. Stem-like CD8 T cells (Tpex) are capable of slow self-renewal and serve as precursors providing proliferative bursts of effector CD8 T cells upon PD-1 blockade therapy, making them an important therapeutic target for effective antiviral and anticancer immunotherapy. IL-7 is a homeostatic cytokine critical for the maintenance of naïve and memory T cells through induction of proliferative and pro-survival signals. During chronic viral infection, the IL-7 receptor (CD127; IL-7Rα) is preferentially expressed by TCF1⁺ stem-like virus-specific CD8 T cells as opposed to the TCF1⁻ terminally differentiated subset. In this study, using NT-I7, a long-acting hybrid Fc-fused recombinant human IL-7 (rhIL-7-hyFc, efineptakin alfa), we demonstrate that IL-7 treatment can selectively expand the crucial stem-like CD8 T cell population. Single-cell gene profile analysis revealed a unique cluster of proliferating TCF1⁺ CD127⁺ cells induced by NT-I7 treatment. This cluster exhibited enrichment of a stem-like signature, including transcription factors preferentially expressed in stem-like cells and costimulatory molecules. They also displayed high levels of genes associated with the cell cycle and lymphocyte migration but low levels of gene encoding effector molecules and multiple inhibitory receptors. While stem-like cells preferentially reside in T cell zones of lymphoid organs and are rare in circulation, we observed the stem-like CD8 T cells were increased in circulating blood and peripheral tissues following NT-I7 treatment. Our data suggest the potential of NT-I7 to enhance antiviral and anticancer immunotherapies by amplifying the crucial stem-like CD8 T cell pool and mobilizing them to the peripheral target sites.

Introduction

Differentiation of Ag-specific CD8 T cells during chronic viral infection



Stem-like/ progenitor(Tpex)

PD-1⁺ TCF1⁺ Bcl6⁺
↑ Costimulatory molecules
↓ Inhibitory receptors
↓ Effector molecules
Quiescent (slow self-renewal)
Proliferative potential
Lymphoid tissues

Transitory effector

↓ TCF1
↑ Tim-3
↑ T-bet, Ki67⁺
↑ Effector molecules
Circulating

Terminally Exhausted

PD-1⁺ TCF1⁻ Blimp1⁺
↑ Costimulatory molecules
↑ Inhibitory receptors
↑ Effector molecules
Sites of infection.
Non-lymphoid and lymphoid tissues

- TCF1⁺ stem-like (progenitor) CD8 T cell subset provide a proliferative burst of effector CD8 T cells upon PD-1 blockade therapy. Im, Nature (2016), Utzschneider, Immunity (2016), M. Sade-Feldman, Cell (2018)
- Frequency of TCF1⁺ stem-like CD8 T cells in the tumor correlate with outcome of PD-1 blockade therapy. Siddiqui, Immunity (2019)

Chronic LCMV infection model

- Transient depletion of CD4 T cells (GK1.5) during LCMV CL-13 infection establishes life-long multiorgan viremia leading to profound CD8 T cell exhaustion. Matloubian, J Virol (1994), Zajac, J Exp med (1994)

NT-I7 (efineptakin alfa, hybrid Fc-fused recombinant human IL-7)

- Less steric hindrance due to flexible IgD hinge
- Long acting (IgG4, FcRn-mediated recycling)
- No antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) Kim J. Clin Tranl Immunology (2020)

Results

Expression of CD127 in LCMV-specific CD8 T cell subsets during chronic LCMV infection

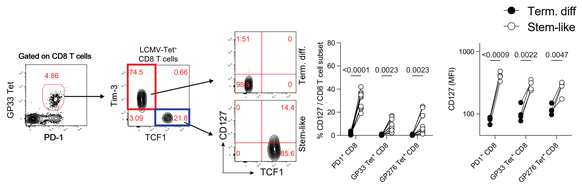


Figure 1. Expression of CD127 in the subsets of LCMV-specific CD8 T cells during chronic LCMV infection. CD4-depleted C57BL/6 mice were infected with LCMV Clone 13. Splenocytes were isolated and analyzed by flow cytometry > 45 days post infection. Frequency of CD127⁺ cells and MFI of CD127 in stem-like (TCF1⁺Tim3⁻) and terminally differentiated (TCF1⁻Tim3⁺, TD) subsets of LCMV-specific H-2Dᵇ GP33 and GP276 Tet⁺ CD8 T cells and PD-1⁺ CD8 T cells in the spleen. A paired t-test was performed to determine p-value.

Expansion of total CD8 and CD4 T cells by NT-I7

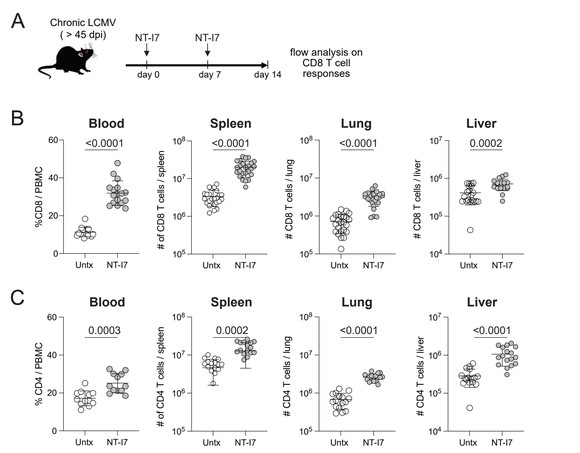


Figure 2. Expansion of total CD8 and CD4 T cells by NT-I7.
(A) Experimental set up of NT-I7 treatment in chronically infected mice. CD4-depleted C57BL/6 mice were infected with LCMV Clone 13. > 45 days post infection, mice were treated with two doses of injection buffer, or 200 μg NT-I7 subcutaneously (s.c.) with a one-week interval.
(B) Frequency or number of total CD8 T cells in blood, spleen, lung, and liver.
(C) Frequency or number of total CD4 T cells in blood, spleen, lung, and liver.
Data represent the mean and SD. An unpaired t-test was performed on the frequency of the cells and log-transformed number of cells.

NT-I7 selectively induces expansion of LCMV-specific TCF1+ CD8 T cell subset during chronic LCMV

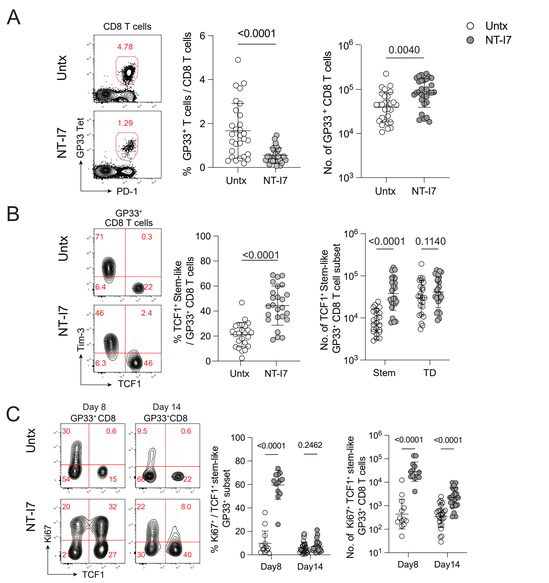


Figure 3. Effect of NT-I7 on LCMV-specific CD8 T cell subsets in the spleen during chronic infection.
(A) Frequency and number LCMV-specific H-2Dᵇ GP33 Tet⁺ CD8 T cells.
(B) Frequency and number of stem-like (TCF1⁺Tim3⁻) and terminally differentiated (TD, TCF1⁻Tim3⁺) GP33 Tet⁺ CD8 T cells.
(C) Expression of Ki67 (proliferation marker) on LCMV-specific GP33 Tet⁺ CD8 T cells in the spleen.
mean and SD. An unpaired t-test was performed on frequency of the cells and log-transformed number of cells.

Single cell gene profile of LCMV-specific CD8 T cells after NT-I7 treatment

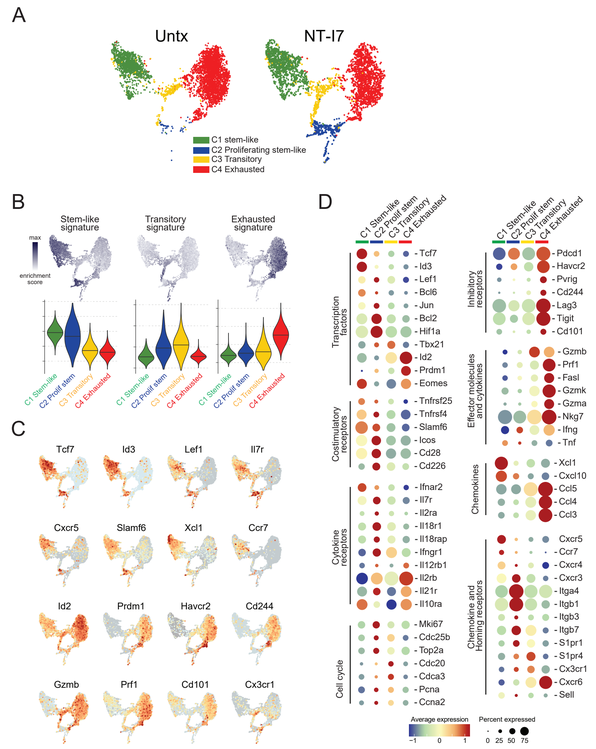


Figure 4. scRNA sequencing on LCMV-specific CD8 T cells. Chronically infected mice were treated with a single dose of injection buffer, or 200 μg NT-I7 (s.c.). Single-cell RNA sequencing was performed on sorted GP33 Tet⁺ splenic CD8 T cells at day 8 of NT-I7 treatment.
(A) UMAP clustering of single-cell transcriptional profiles of GP33 Tet⁺ CD8 T cells from control and NT-I7 treated mice.
(B) VISION analyses of each cluster for enrichment of gene signatures associated with LCMV-specific stem-like, transitory, and exhausted CD8 T cells. UMAP plots show the cells enriched for each gene signature. Violin plots shows the enrichment of genes signatures for each cluster.
(C) Gene expression of selected genes in single cells.
(D) Average gene expression and percentage of expressed of selected genes.

Spatial distribution of virus-specific TCF1⁺ CD8 T cells within the spleen after NT-I7 treatment

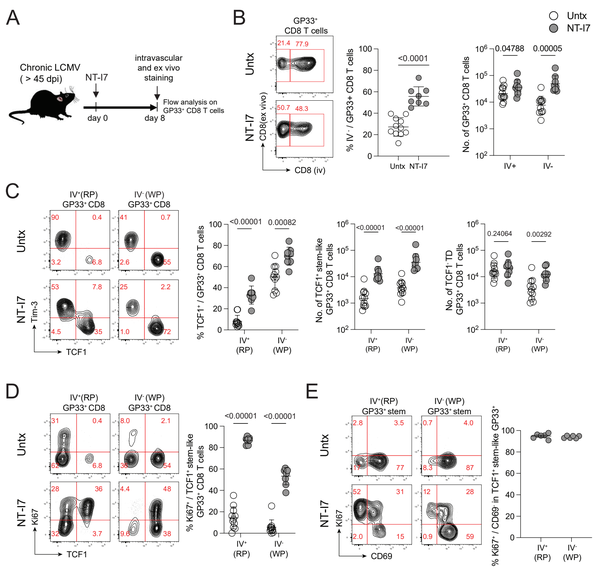


Figure 5. LCMV-specific GP33 Tet⁺ CD8 T cell subsets in the white and red pulp.
(A) Experimental set up of NT-I7 treatment in chronically infected mice. Chronically mice were treated with a single dose of injection buffer, or 200 μg NT-I7 (s.c.). At day 8, splenocytes were isolated after intravascular labeling and analyzed by flow cytometry.
(B) Frequency and number of IV⁺ (red pulp) and IV⁻ (white pulp) GP33 Tet⁺ CD8 T cells
(C) Frequency and number of stem-like and TD subset of LCMV GP33-specific CD8 T cells in IV⁺ (RP) and IV⁻ (WP) compartments.
(D) Frequency of Ki67⁺ cells in stem-like GP33 Tet⁺ CD8 T cells in IV⁺ (RP) and IV⁻ (WP) compartments.
(E) Expression of Ki67 and CD69, in TCF1⁺ GP33 Tet⁺ CD8 T cells in the IV⁺ (RP) and IV⁻ (WP) compartments.
Data represent the mean and SD. An unpaired t-test was performed on the frequency of the cells and log-transformed number of cells.

Increase of LCMV-specific TCF1⁺ CD8 T cells in the peripheral blood by NT-I7 treatment

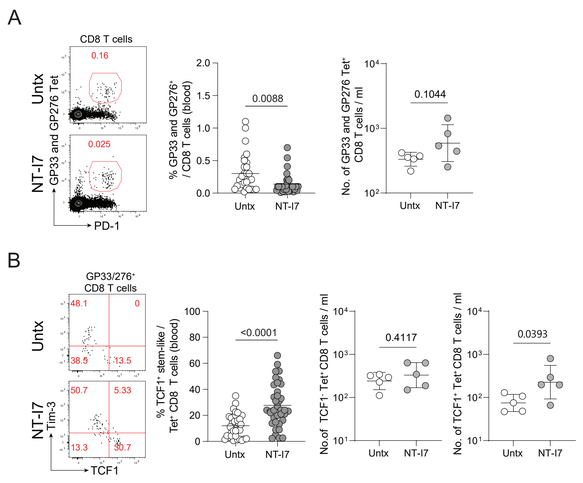


Figure 6. LCMV-specific TCF1⁺ CD8 T cells in the peripheral blood.
Chronically infected mice were treated with two doses` of injection buffer, or 200 μg NT-I7 (s.c.) with a one-week interval and PBMCs were isolated and analyzed by flow cytometry at day 14.
(A) Frequency of LCMV-specific GP33 and GP276 Tet⁺ CD8 T cells.
(B) Frequency of the stem-like subset in GP33 and GP276 Tet⁺ CD8 T cells.
Flow plots are gated on CD8 T cells (A), or LCMV-specific GP33 and GP276 Tet⁺ CD8 T cells (B) as indicated. Data represent the mean and SD. An unpaired t-test was performed on the frequency of the cells and log-transformed number of cells.

Increase of LCMV-specific TCF1⁺ CD8 T cells in the peripheral tissues by NT-I7 treatment

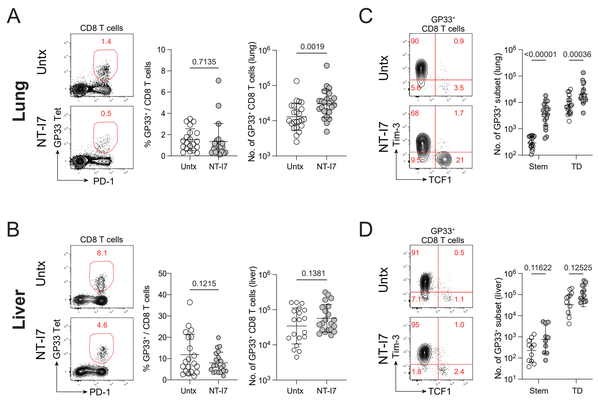


Figure 7. LCMV-specific TCF1⁺ CD8 T cells in non-lymphoid tissues.
Chronically infected mice were treated with two doses of injection buffer, or 200 μg NT-I7 (s.c.) with a one-week interval and lymphocytes were isolated and analyzed by flow cytometry at day 14.
(A and C) Frequency and number of LCMV-specific GP33⁺ CD8 T cells in the lung and liver.
(B and D) Frequency and number of the stem-like subset in GP33 Tet⁺ CD8 T cells in the lung and liver.

Conclusions

- NT-I7 treatment in chornically infected mice preferentially induces expansion of LCMV-specific TCF1⁺ CD8 T cell subset.
- Proliferating TCF1⁺ CD8 T cell population induced by NT-I7 displays TCF1⁺ stem-like signature and migratory features.
- NT-I7 treatment increases TCF1⁺ CD8 T cells in the circulation and non-lymphoid peripheral tissues.

Acknowlegements

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