Cytolytic HIV-specific CD8+ T cells do not recirculate through tissues

Marcus Buggert[1,2], Son Nguyen[2], Samuel Darko[3], Amy Ransier[3], Gustavo-Reyes Terán[4], Daniel C. Douek[3], Yoav Dori[5], Ian Frank[6], Max G. Itkin[5], Michael R. Betts[2]

1) Center of Infectious Medicine, Dept. of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden. 2) Dept. of Microbiology, University of Pennsylvania, Philadelphia, PA, USA. 3) Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. 4) Dept. de Investigación en Enfermedades Infecciosas, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico. 5) Children's Hospital of Philadelphia, Penn Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA , USA. 6) Div. of Infectious Diseases, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Our results demonstrate that not all types of memory CD8+ T cells survey tissues and reveal that cytolytic molecule expression is mostly confined to effector memory HIV-specific CD8+ T cells in blood during steady-state and chronic HIV disease.

Introduction

➢Cytolytic effector memory HIV- and SIV-specific CD8+ T cells are key correlates of natural and vaccine-induced lentiviral control.

➢However, current knowledge of HIV-specific CD8+ T cells relies almost entirely on circulating cells from peripheral blood, although HIV is a disease primarily affecting lymphoid tissues.

➢Recent publications have demonstrated low abundance of cytolytic HIV-specific CD8+ T cells in lymphoid tissues and gut.

➢As such, it remains unknown if cytolytic effector memory HIV-specific CD8+ T cells leave the blood to access HIV reservoirs in lymphoid and peripheral tissues.

Aim

To directly assess if effector memory CD8+ T cells are able to recirculate and egress from tissues through access to HIV-infected thoracic duct lymph (TDL)

Material and methods

➢ Human peripheral blood, lymph nodes and matched TDL mononuclear cells were collected through thoracic duct cannulation from of HIV-seronegative and seropositive individuals on and off antiretroviral therapy in North America. Other types of peripheral organs, including liver, endometrium and gut (colon), were also collected from HIV-seronegative individuals in Europe.

➢ Multi-parametric flow cytometry, RNA-seq (SMART-Seq2), TCR-seq and tetramer analyses were used to assess the functional, phenotypic, and transcriptional profile of CD8+ T cells.

➢ Results were analyzed using RStudio, FlowJo, and GraphPad Prism. * P < 0.05; ** P < 0.01; *** P < 0.001.

Karolinska Institutet

Marcus Buggert, MSc, PhD
Assistant Professor
Dept. of Medicine Huddinge
Karolinska Institutet, Stockholm, Sweden
E-mail: marcus.buggert@ki.se

Results

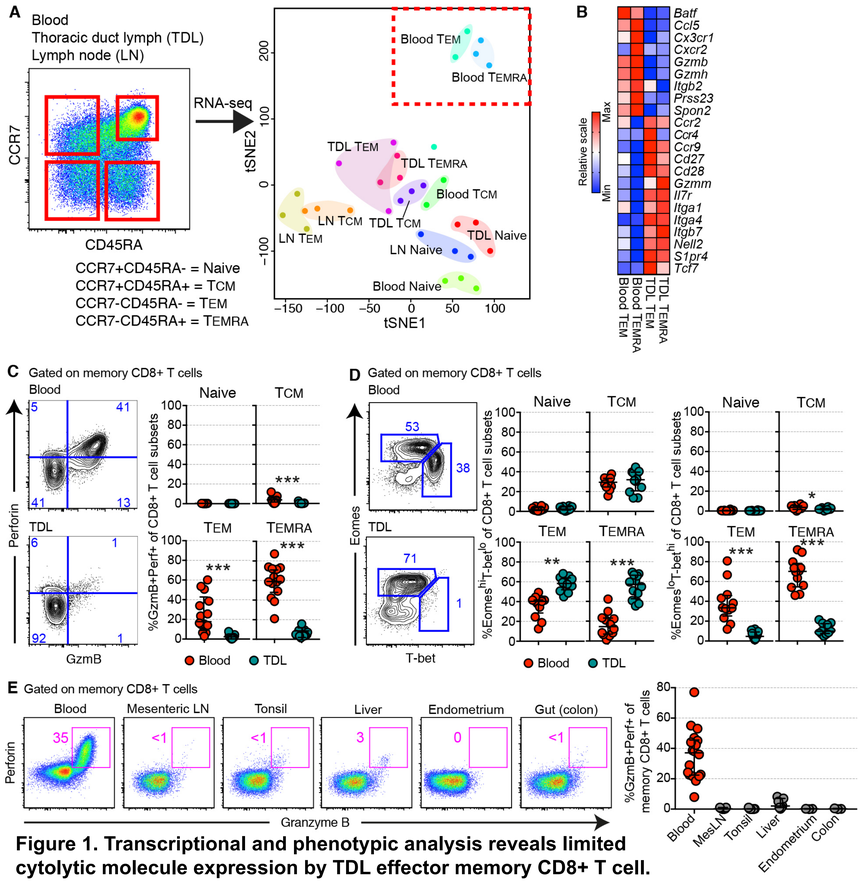


Figure 1. Transcriptional and phenotypic analysis reveals limited cytolytic molecule expression by TDL effector memory CD8+ T cell.

(A) RNA-seq analysis of blood, TDL and LN CD8+ T cells populations and corresponding tSNE plot. **(B)** Selected set of differentially expressed genes for blood and TDL effector memory populations. Differential expression of (**C**) cytolytic molecules (**D**) transcription factors between blood and TDL CD8+ T cells. (**E**) Cytolytic molecule expression is limited in multiple tissues compared to blood memory CD8+ T cells.

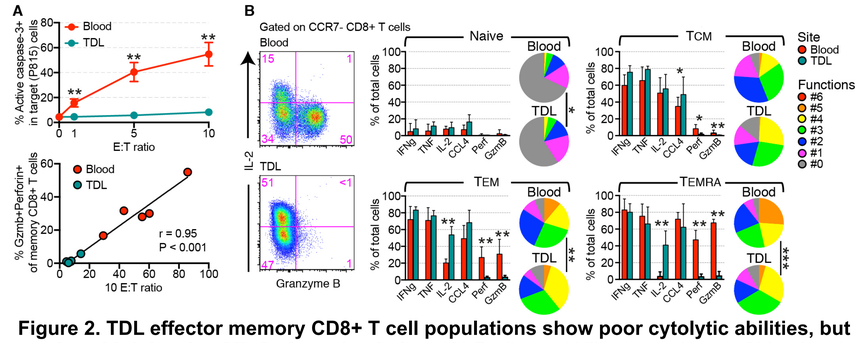


Figure 2. TDL effector memory CD8+ T cell populations show poor cytolytic abilities, but produce high levels of IL-2 after stimulation. (A) Redirected killing experiment of blood and TDL CD8+ T cells (top) and correlation to cytolytic molecule expression. **(B)** Functional analysis (after PMA-Ionomycin stimulation) between blood and TDL CD8+ T cell populations.

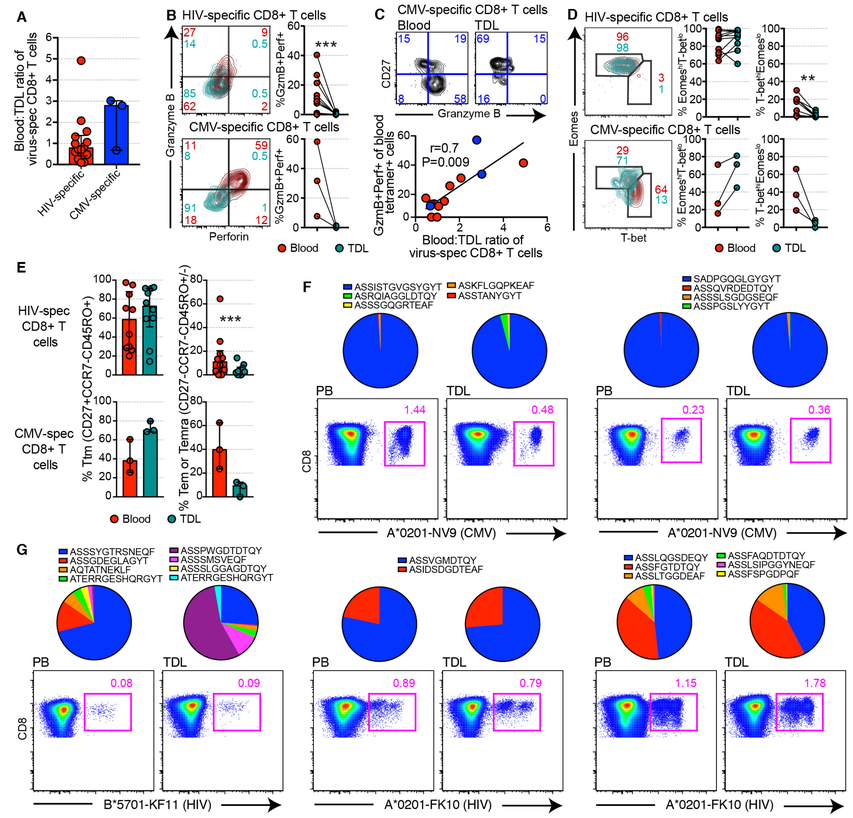


Figure 3. The majority of tissue egressing (TDL) HIV- and CMV-specific CD8+ T cells are non-cytolytic. (A) The blood:TDL ratio of HIV- and CMV-specific CD8+ T cells based on tetramer stainings. **(B)** Cytolytic molecule expression between virus-specific CD8+ T cells in blood and TDL. **(C)** Typical phenotype of CMV-specific CD8+ T cells in blood vs. TDL (top). Correlation between the blood:TDL ratio of virus-specific CD8+ T cells and cytolytic molecule expression by the same cells (bottom). **(D)** Expression pattern of the T-box transcription factors T-bet and Eomes between blood and TDL HIV- and CMV-specific CD8+ T cells. **(E)** Phenotype distribution of virus-specific CD8+ T cells between blood and TDL. **(F)** CMV- and **(G)** HIV-tetramer+ CD8+ T cell clonotype distribution between blood and TDL. Every single point represent a tetramer+ response.

Conclusions

➢ Transcriptional and functional profiling shows that expression of cytolytic molecules are confined to blood memory CD8+ T cells.

➢ Instead, phenotypic CD8 counterparts in TDL, and many tissues, represent non-cytolytic cells with high IL-2 production.

➢ Tissue egressing HIV-specific CD8+ T cells lack cytolytic activity.

➢ The maturation status (CCR7-CD45RA-CD27+) of HIV-specific CD8+ T cells is a phenotypic fingerprint of recirculating T cells.