Lymphoid TCF1$^+$CD39$^+$ CD8$^+$ T cells maintain stem-like features and contribute to viral control

Improved understanding of CD8$^+$ T cell function during HIV infection is vital to designing an HIV cure. We have identified a subset of lymph node CD8$^+$ T cells that demonstrate simultaneous stem-like and effector properties and are strongly associated with viral control during SIV and HIV infection.

This is a summary of:
Strongin, Z. et al. Distinct SIV-specific CD8+ T cells in the lymph node exhibit simultaneous effector and stem-like profiles and are associated with limited SIV persistence. *Nat. Immunol.* https://doi.org/10.1038/s41590-024-01875-0 (2024).

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The question

Much of our understanding of CD8$^+$ T cell functionality, stemness and exhaustion in chronic antigen settings comes from work in lymphocytic choriomeningitis virus (LCMV) and cancer. This work has identified TCF1$^+$CD8$^+$ T cells as the stem-like population that fuels the differentiated effector and exhausted CD8$^+$ T cell populations, typically identified by lack of TCF1 expression and high expression of inhibitory receptors and markers of terminal differentiation, including CD39[1,2]. To design better T cell therapeutic approaches for HIV, we aimed to understand the kinetics and relevance of these same CD8$^+$ T cell subsets after HIV infection. In addition, we wanted to explore this question with a particular focus on lymph node CD8$^+$ T cells, given the role of lymph nodes as primary sites of viral replication and previous demonstrations of unique CD8$^+$ T cell biology within lymph nodes[3,4].

The observation

To investigate CD8$^+$ T cell dynamics in lymph nodes after HIV infection and considering the limitations of obtaining lymph nodes from people living with HIV (PLWH), we took advantage of the rhesus macaque SIV infection model, which enabled well-controlled lymph node biopsies at varying timepoints after infection. Using high parameter flow cytometry, we were able to identify a TOX^{hi}TCF1$^+$CD39$^+$CD8$^+$ T cell population, unique in its high expression of markers of both stemness and terminal differentiation, while lacking expression of canonical cytotoxic molecules. We demonstrated that these cells are responsive to SIV peptides and further characterized their simultaneous stem-like and effector profile using transcriptomic and proteomic analyses. In addition, we utilized RNAscope in combination with hiplex immunohistochemistry to interrogate the localization of TOX$^+$TCF1$^+$CD39$^+$CD8$^+$ T cells within follicular microenvironments.

We found that TOX^{hi}TCF1$^+$CD39$^+$CD8$^+$ T cells, with a unique phenotypic, functional and transcriptional profile, were strongly associated with improved control of plasma viremia and limited tissue viral burden (Fig. 1). Furthermore, TCF1$^+$CD39$^+$CD8$^+$ T cells proliferated in response to immune checkpoint blockade (including PD-1 blockade) and fueled the differentiation of terminal effector CD8$^+$ T cells. These cells were preferentially located within B cell follicles, a site of high viral replication with the potential to serve as an immune sanctuary, and were found to be in closer proximity to SIV$^+$ CD4$^+$ T cells than SIV$^-$ CD4$^+$ T cells.

In addition to SIV-infected macaques, we investigated these same CD8$^+$ T cell dynamics in lymph nodes from antiretroviral therapy (ART)-naive and ART-suppressed PLWH and found that TOX^{hi}TCF1$^+$CD39$^+$CD8$^+$ T cells with a highly similar phenotype are also expanded in PLWH and associated with lower intact reservoir size in ART-suppressed PLWH.

The implications

This study provides insight into the dynamics of canonical stem-like and terminally differentiated CD8$^+$ T cells in lymphoid tissue, and identifies a previously undescribed population of TCF1$^+$CD39$^+$CD8$^+$ T cells that are associated with viral control despite a lack of expression of canonical cytotoxic molecules traditionally associated with antigen responsiveness. These findings also highlight the value of exploring these cell populations across diverse diseases, as the populations we describe and their kinetics after infection seem distinct from those reported in LCMV and cancer studies.

A limitation to this work is that we were unable to fully interrogate the functional capacity of TCF1$^+$CD39$^+$CD8$^+$ T cells given the inability to sort live TCF1$^+$ cells and, therefore, we could not demonstrate a direct ability of these cells to limit virally infected cells. In addition, given the difficulty in obtaining lymph node biopsies from PLWH, obtaining a high enough sample number from a consistent collection timepoint to demonstrate an association of the frequency of TCF1$^+$CD39$^+$CD8$^+$ T cells with plasma viremia in ART-naive PLWH was challenging.

Our observations highlight an opportunity to explore the dynamics of TCF1$^+$CD39$^+$CD8$^+$ T cells at additional stages of infection as well as in other tissue sites. Furthermore, investigations into the ability of these cells to promote responsiveness to therapeutic HIV interventions could valuably inform future study designs. Finally, interventions that can expand this cell subset in lymphoid tissues might prove of crucial importance for curative HIV strategies.

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EXPERT OPINION

"Strongin et al. have identified a TOX-expressing CD8⁺ T cell population associated with control of SIV infection in lymphoid tissue of rhesus macaques. The frequency of these cells was strongly associated with viral control and lower SIV reservoir size. The authors recapitulated findings in human samples and conclude that these cells represent a lymphoid CD8⁺ T cell subset with both stem-like and effector properties that contribute to limiting virus persistence." **Daniel Douek, NIH, Bethesda, MD, USA.**

FIGURE

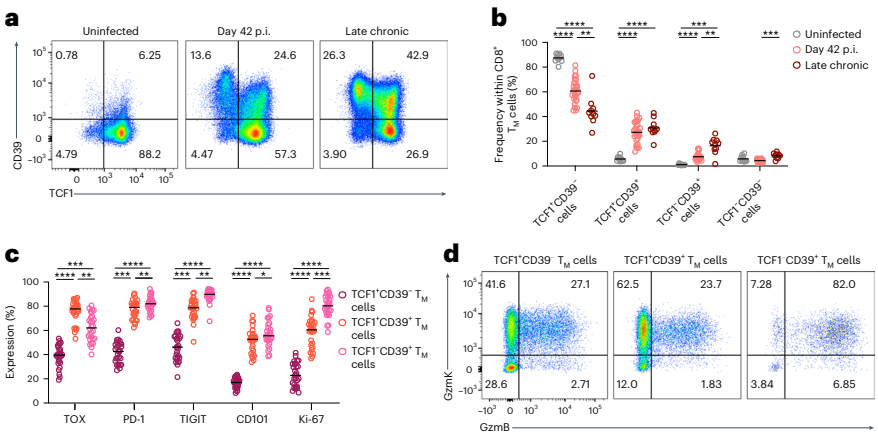

Fig. 1 | TCF1⁺CD39⁺CD8⁺ T cells expand after SIV infection. a,**b**, Representative staining of TCF1 and CD39 (**a**) and frequency of TCF1⁺CD39⁻, TCF1⁺CD39⁺ and TCF1⁻CD39⁺ cells within CD8⁺ memory T (T_M) cells (**b**) in the lymph nodes of SIV-infected macaques at day 42 post infection (p.i.) ($n = 28$) or month 17 p.i. (mean, late chronic) ($n = 10$) or left uninfected ($n = 10$). **c**, Expression of phenotypic markers of interest in TCF1⁺CD39⁻, TCF1⁺CD39⁺ and TCF1⁻CD39⁺ subsets of CD8⁺ T_M cells in the lymph nodes of SIV-infected macaques at day 42 p.i. ($n = 28$). **d**, Representative staining of GzmB and GzmK among TCF1⁺CD39⁻, TCF1⁺CD39⁺ and TCF1⁻CD39⁺ subsets of lymph node CD8⁺ T_M cells, as in **c**. *P* values determined by two-way ANOVA with Tukey's multiple comparisons test (**b**) or Friedman test one way-ANOVA with Dunn's multiple comparisons (**c**). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. © 2024, Strongin, Z. et al.

BEHIND THE PAPER

Emory University is home to cutting-edge research in basic CD8⁺ T cell biology and cancer. We set out to apply the findings of our colleagues to the HIV cure space, expecting to find similar dynamics of stem-like and exhausted CD8⁺ T cell populations, with the goal of maximizing our ability to access tissue samples using the macaque model. We were surprised to find this previously undescribed cell population, identified by markers typically singularly expressed in either stem-like (TCF1) or terminally differentiated (CD39) cells. Taking advantage of a large network of specialists across the HIV and CD8⁺ T cell fields (eight unique contributing groups), we sought to comprehensively profile these unique cells and their relationship with viral control. **Z.S. & M.P.**

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FROM THE EDITOR

"CD8⁺ T cells are crucial to control HIV and the lymph node is a primary site of viral replication and persistence. As such, the identification of a population of lymph node CD8⁺ T cells associated with low plasma viremia and a reduced SIV reservoir in macaques, and which retain stem-like properties, although they have hallmarks of effector T cells, is of high relevance." **Ioana Staicu, Senior Editor, *Nature Immunology*.**