A Closer Look at HIV-Specific T-cells in Mucosal Tissues

Shacklett Laboratory
University of California
Davis, CA USA

Outline

Why does the cellular immune response fail to clear mucosal HIV infection?

1) *Might earlier/stronger mucosal CD8+ T-cell responses be beneficial (or harmful)?*
2) *What is the "balance" between cytotoxic capacity and cytokine polyfunctionality?*
3) *Does immune exhaustion play a role in the failure to clear HIV from mucosal tissues?*

Mucosal CD8+ T-cells: Too Little, Too Late?

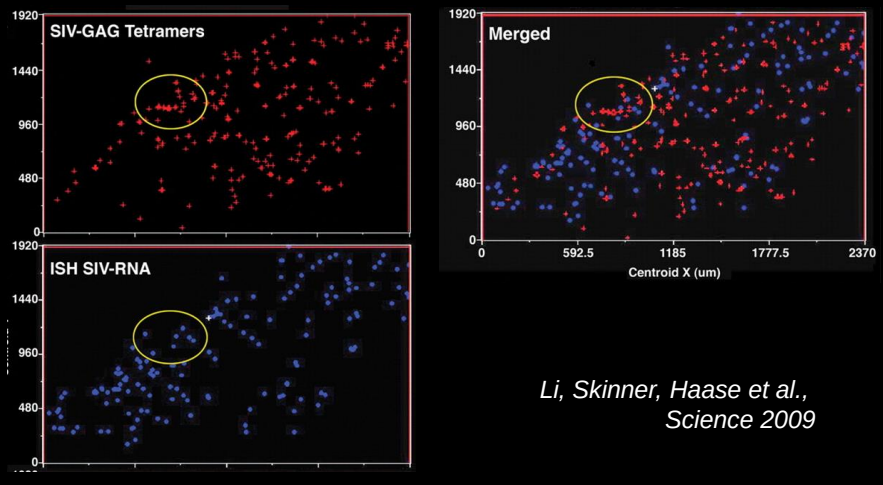
JOURNAL OF VIROLOGY, July 2005, p. 9228–9235 Vol. 79, No. 14
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.14.9228–9235.2005

CD8$^+$ T-Lymphocyte Response to Major Immunodominant Epitopes after Vaginal Exposure to Simian Immunodeficiency Virus: Too Late and Too Little

Matthew R. Reynolds,[1,2] Eva Rakasz,[1] Pamela J. Skinner,[3] Cara White,[3]† Kristina Abel,[4,5,6] Zhong-Min Ma,[4,5,6] Lara Compton,[4,5,6] Gnankang Napoé,[1] Nancy Wilson,[1] Christopher J. Miller,[4,5,6] Ashley Haase,[7]* and David I. Watkins[1,2]*

- **SIVmac model: Responses in mucosal tissues lagged behind the development of viremia following vaginal challenge**
- **Notably, SIV-specific CD8+ T-cell responses in gut were significantly delayed, likely contributing to the massive CD4+ T-cell depletion observed in the gut**
- ***"Too Little, Too Late" implies that "more, sooner" would be better (→ mucosal vaccination)***

"The Battlefield": Host-vs-Virus conflict

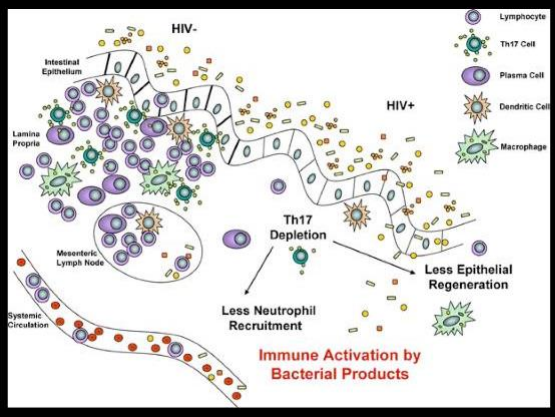


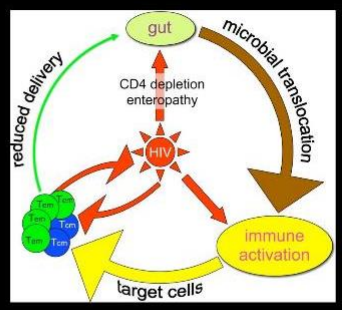
Li, Skinner, Haase et al., Science 2009

In vivo effector:target ratio determines outcome

However, other work suggests a potential role for CD8+ T-cells in mucosal barrier compromise

Early Events in the GI Tract





A "Vicious Cycle"

Hofer and Speck, 2009; Douek et al., 2009

Early Events in the GI Tract

- **Depletion of CD4+ T-cells, particularly Th17**

→ **Leads to intestinal barrier compromise**
(Brenchley, Douek)

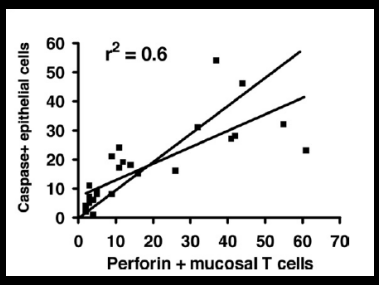
→ **Increased microbial translocation**

→ **Generalized immune activation**

- ***Infiltration and/or expansion of CD8+ T-cells, inversion of CD4:CD8 ratio in gut***
  - ***Could this conceivably contribute to barrier compromise?***

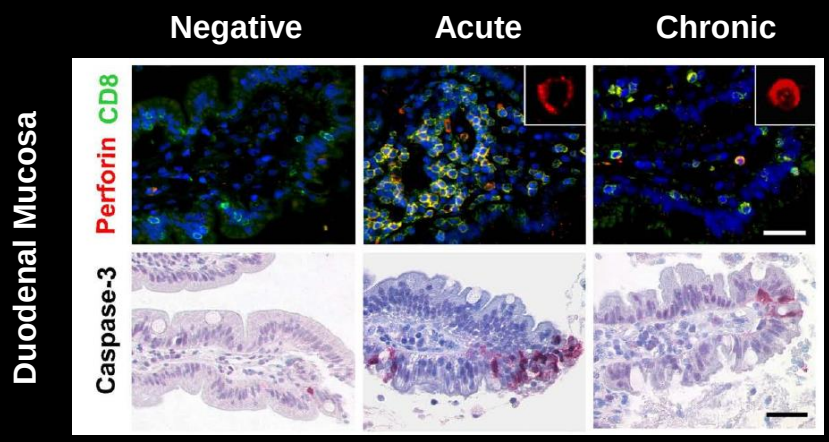
A Role for CD8+ T-cells in Barrier Compromise During Acute Infection?

- ***"Before depleting CD4+ T-cells, acute HIV infection induces <u>infiltration</u> of the mucosa with activated effector CD8+ T-cells. The HIV-induced barrier defect…might arise from increased epithelial apoptosis, induced by perforin-positive mucosal cytotoxic T cells."***



Epple et al., Gastroenterology 2010

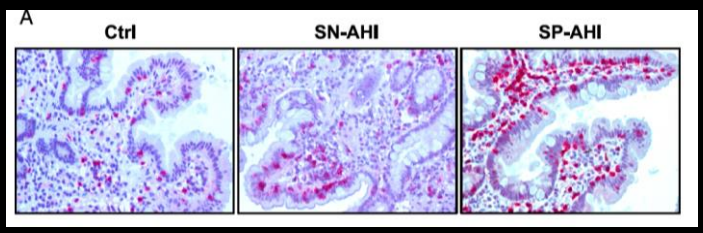
A Role for CD8+ T-cells in Acute Stage Gut Epithelial Barrier Damage?



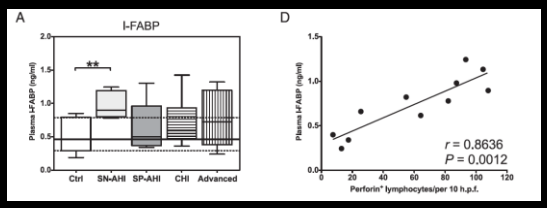
Epple et al., Gastroenterology 2010

Perforin+ CD8+ T-cells Are Recruited From Periphery During Acute Infection

Pre-SC AHI *Post-SC AHI*

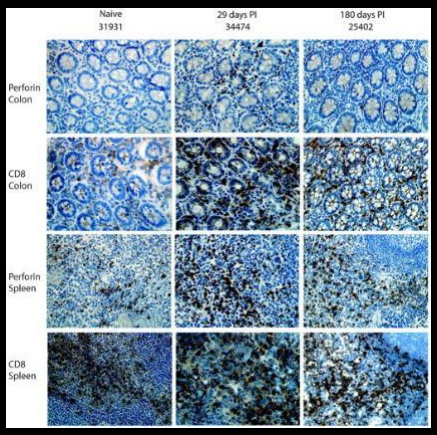


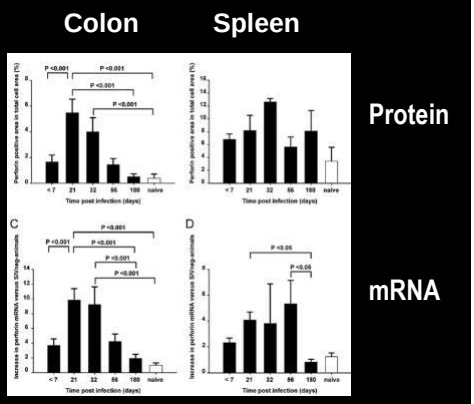
- **Perforin correlates with plasma I-FABP, a marker of immune activation**



Allers et al., JI 2017

In SIVmac, perforin expression in colorectal CD8+ T-cells is limited to acute infection





- **Gut perforin may be tightly regulated to preserve barrier integrity and limit 'collateral damage'**

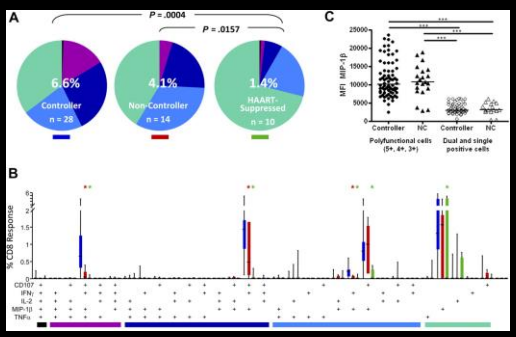
Quigley et al., J. Virol. 2006

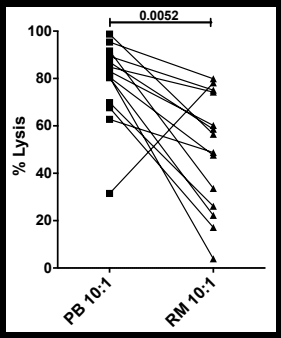
2) What is the "balance" between cytotoxic capacity and cytokine polyfunctionality?

- ***Cytotoxicity vs cytokine polyfunctionality?***
- ***Strong polyfunctional responses are detected in HIV Controllers***
- ***BUT low perforin expression and weak cytotoxicity are observed in all groups***

What is the balance between cytotoxic capacity and cytokine polyfunctionality?

- *Strong polyfunctional responses in Controllers; low perforin expression and weak cytotoxicity in all groups*





Ferre et al., Blood 2009

Kiniry et al., Mucosal Immunology 2017

What are the mucosal expression patterns of other cytotoxic effector proteins?

GRANZYMES: Serine proteases found in cytotoxic granules
- **GrzA: caspase-independent cell death**
- **GrzB: caspase-dependent cell death (cleaves caspase-3)**

Both have a possible role in degrading extracellular matrix

"Orphan" Granzymes:
- **GrzK: gene duplication of GrzA; proinflammatory (monocyte activation)**
- **GrzM: present in NK and γδ T cells**
- **GrzH: possible caspase-independent cell death**

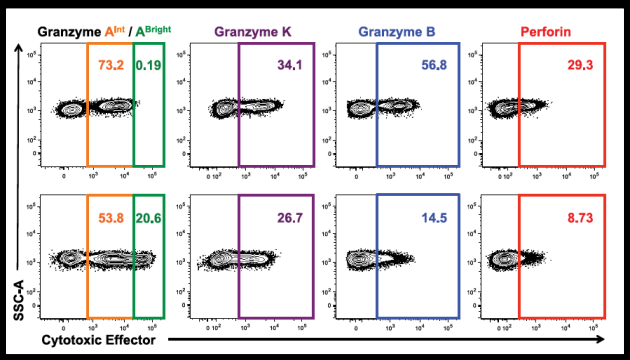
Granulysin: Killing of intracellular bacteria (Lieberman 2014)

Granzyme/Perforin expression varies between tissues…

(All CD8+ T-cells)

Blood

Gut

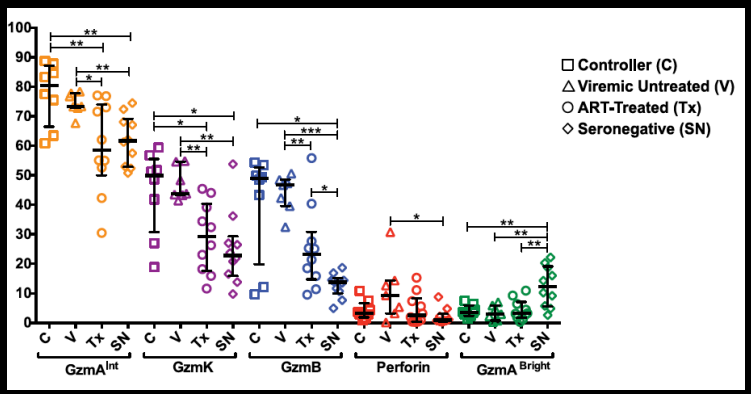


- *Higher expression of granzymes and perforin in blood vs gut*
- *Also true at mRNA level (not shown)*
- *Novel Granzyme A^{high} subset present only in gut*

Kiniry et al., J Immunol 2018

…and also varies between participant groups

% Gut CD8+ T-cells (all specificities)

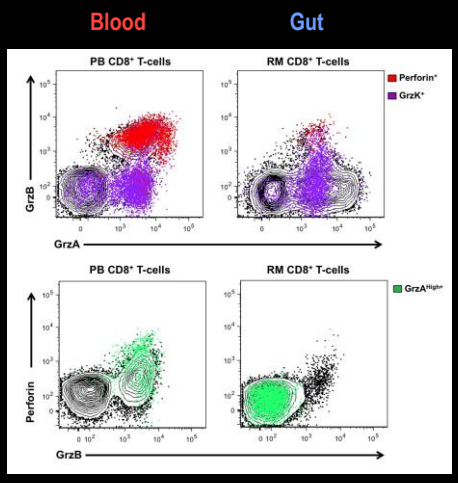


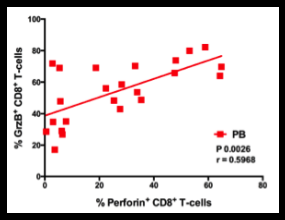
- *Note relatively low perforin, high GzmA/B in Controllers and Viremic untreated participants;*
- *High GzmA^{bright} in Seronegatives*

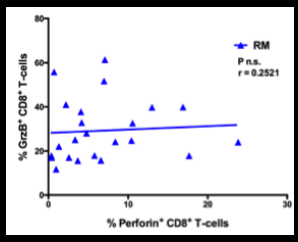
Kiniry et al., J Immunol 2018

Co-expression of cytotoxic proteins differs between blood and gut:

Perforin and GrzB expression are strongly correlated in blood, but not in gut

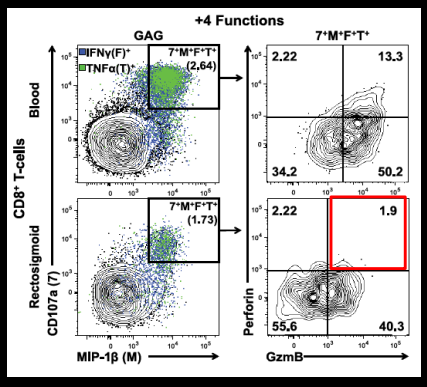


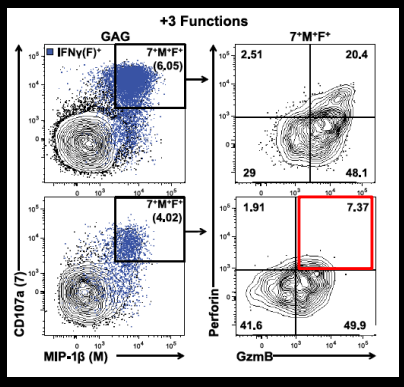




Kiniry et al., J Immunol 2018

In Gut, Cytokine Polyfunctionality Does Not Imply Cytotoxic Capacity

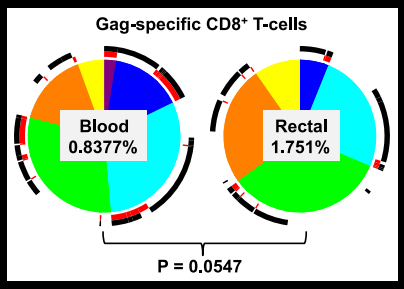


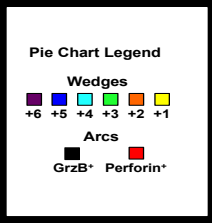


- *In Gut (but not blood), few HIV-specific "polyfunctional" cells express perforin*

Kiniry et al., J Immunol 2018

In gut: apparent <u>dissociation</u> between cytokine polyfunctionality and perforin expression



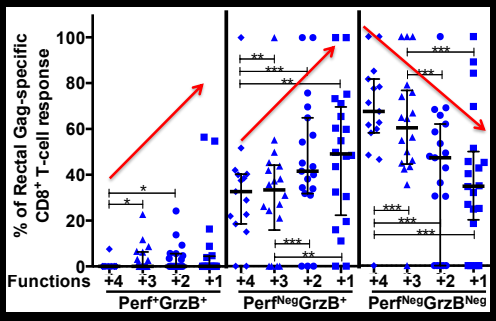


n=27

**Functions tested here: TNFα, IFNγ, MIP-1β, CD107a*

Kiniry et al., J Immunol 2018

In gut: apparent <u>dissociation</u> between cytokine polyfunctionality and perforin expression



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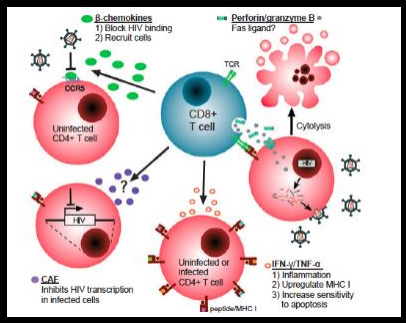
Kiniry et al., J Immunol 2018

Are mucosal CD8+ T-cells primarily *non-cytolytic* effectors?



Or, are there multiple subsets with different functions?

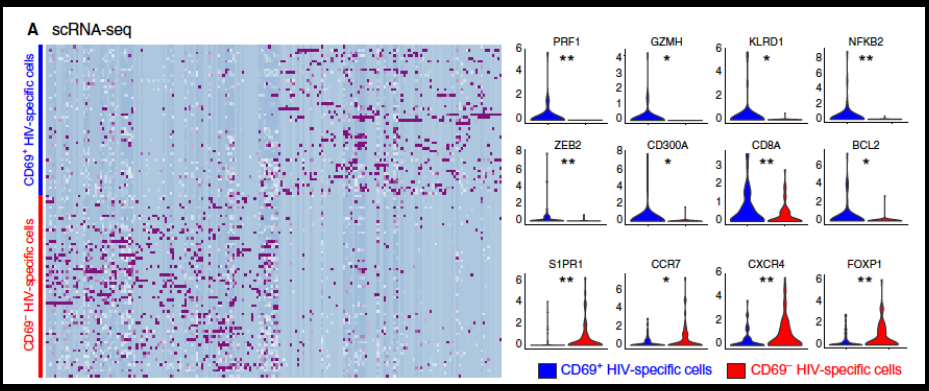
SCIENCE IMMUNOLOGY | RESEARCH RESOURCE

HIV

Identification and characterization of HIV-specific resident memory CD8$^+$ T cells in human lymphoid tissue

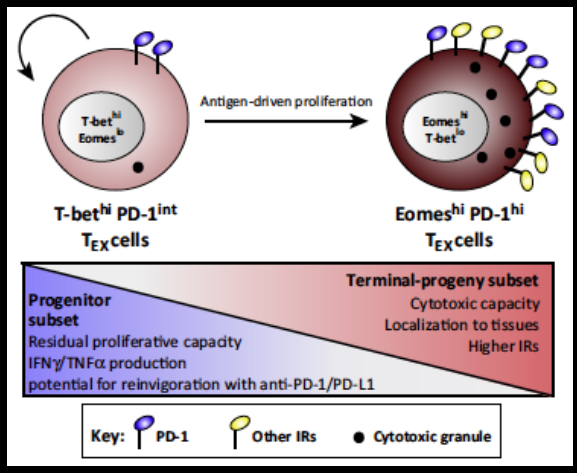
Buggert *et al.*, *Sci. Immunol.* **3**, eaar4526 (2018) 1 June 2018

Within the HIV-specific CD8+ T-cell population, CD69+ and CD69- differ in transcriptional signatures



(3) Does immune exhaustion play a role in the failure to clear HIV from mucosal tissues?

Persistent antigen and chronic inflammation lead to "exhausted" Eomes^{High}/PD1^{High} cells

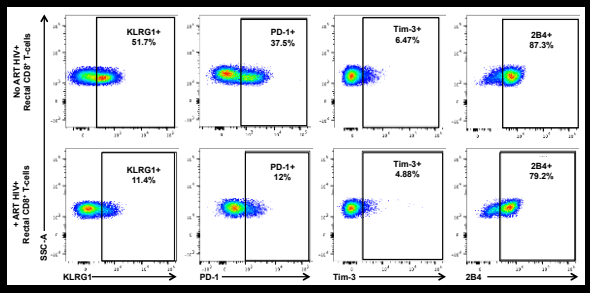


Pauken, K.E. & Wherry, E.J. Trends Immunol. 2015. doi: 10.1016/j.it.2015.02.008

Do some HIV-specific mucosal CD8+ T-cells exhibit characteristics of 'exhaustion'?

NO ART

ART

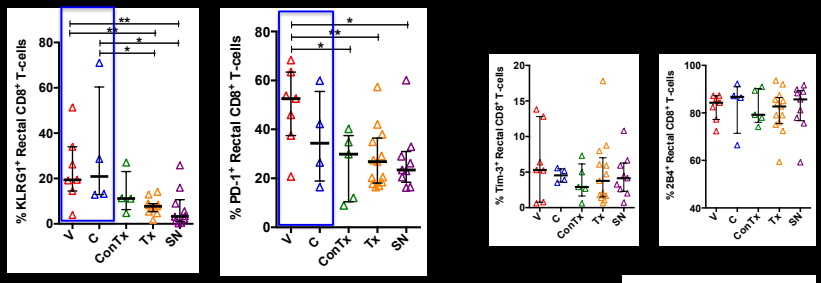


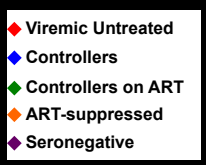
KLRG1 PD1 TIM-3 2B4

Expression of KLRG1 and PD1 is elevated on rectal CD8+ T-cells in viremic and controller groups compared to ART-Tx and seronegative groups.

B. Kiniry, in preparation

Do some HIV-specific rectal CD8+ T-cells exhibit characteristics of 'exhaustion'?

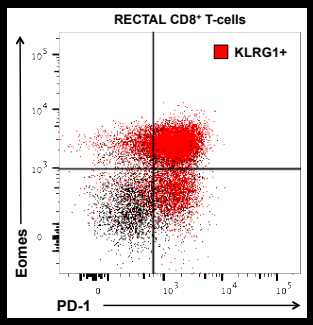


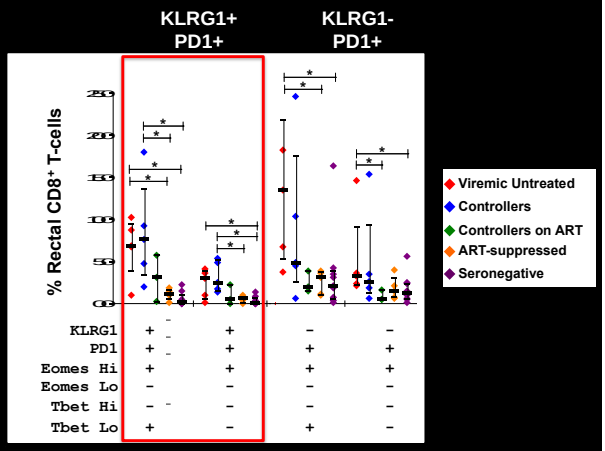


Expression of KLRG1 and PD1, but not Tim3 or 2B4, is elevated on rectal CD8+ T-cells in viremic and controller groups compared to ART-Tx and seronegatives

B. Kiniry, in preparation

KLRG1+/PD1+ mucosal CD8+ T-cells are Eomes^{High}, suggestive of exhaustion

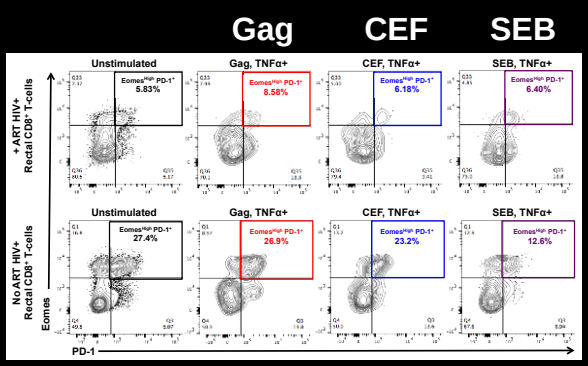


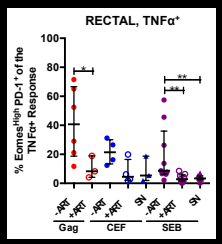


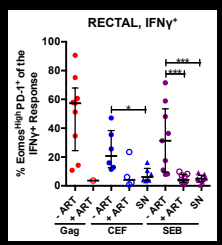
Co-expression of these molecules is high in participants not on ART compared to treated and seronegative subjects

B. Kiniry, in preparation

Antigen-specific gut CD8+ T-cells in untreated HIV infection are skewed towards an Eomes^{High} PD1+ phenotype



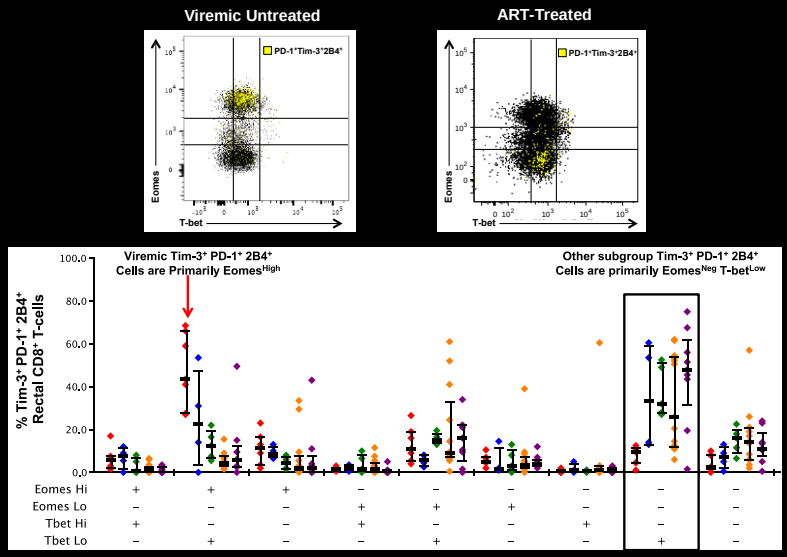




These cells retain some functionality despite a 'terminally exhausted' phenotype.

B. Kiniry, in preparation

Tim3+PD1+2B4+/Eomes^{high} cells are primarily found in viremic individuals not on ART



B. Kiniry, in preparation

Summary

- Gut CD8^{+} T-cells express significantly less perforin and reduced cytotoxic capacity compared to their blood counterparts; however, there is an influx of perforin-expressing CD8+ T-cells during acute infection. Data from one group suggest these cells may contribute to epithelial damage and immune activation.
- Only a small percentage of gut CD8+ T-cells appear to have cytotoxic capacity; however, cytokine polyfunctionality is common particularly in HIV Controllers. Transcriptomics studies may elucidate distinct functional subsets.
- Immune exhaustion appears to be a characteristic of gut CD8+ T-cells in chronic HIV infection; nevertheless, these cells retain some ability to secrete cytokines and degranulate in response to stimulation.

Acknowledgments

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Steve Deeks Peter Hunt Ma Somsouk

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- *Study Volunteers*