TISSUE ISSUES:
Perspectives on HIV-Specific T-cell Responses in Mucosal and Lymphoid Tissues

Shacklett Laboratory
University of California
Davis, CA USA

Acknowledgments

AIDS Memorial Quilt displayed in Washington, DC

We are indebted to our study participants for their generous contributions to this work.

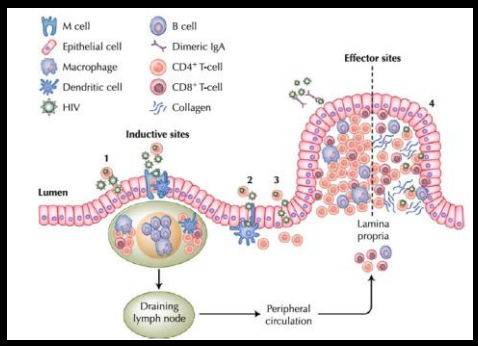
Disclosures

- *Work in my lab is supported by grants from the US National Institutes of Health (NIH), the Bill and Melinda Gates Foundation and the James B. Pendleton Charitable Trust.*
- *Our laboratory has active research contracts with Gilead Sciences, not directly related to the work presented here.*
- *I have served as a consultant to Merck, Inc. within the past year on topics broadly related to those discussed here, but Merck did not fund this work.*

Mucosal Immunity and HIV Infection

Four key aspects:

1) **Defense against HIV acquisition; implications for vaccine development**
2) **Maintenance of barrier integrity; limiting microbial translocation**
3) **Defense against other mucosal pathogens/STIs**
4) **Continuous "battle" within tissues to control viral replication and dissemination throughout chronic infection**



Shacklett B, Anton P, Curr Inf Dis Rep 2010

Gut and Lymph Nodes Contain Most of Viral Burden in HIV/SIV

Defining total-body AIDS-virus burden with implications for curative strategies

Jacob D Estes[1], Cissy Kityo[2], Francis Ssali[2], Louise Swainson[3], Krystelle Nganou Makamdop[4], Gregory Q Del Prete[1], Steven G Deeks[5], Paul A Luciw[6], Jeffrey G Chipman[7], Gregory J Beilman[7], Torfi Hoskuldsson[7], Alexander Khoruts[8], Jodi Anderson[8], Claire Deleage[1], Jacob Jasurda[8], Thomas E Schmidt[8], Michael Hafertepe[8], Samuel P Callisto[8], Hope Pearson[8], Thomas Reimann[8], Jared Schuster[8], Jordan Schoephoerster[8], Peter Southern[8], Katherine Perkey[9], Liang Shang[9], Stephen W Wietgrefe[9], Courtney V Fletcher[10], Jeffrey D Lifson[1], Daniel C Douek[4], Joseph M McCune[3], Ashley T Haase[9] & Timothy W Schacker[8]

Gut: 62% of vRNA pre-ART; 98% post-ART

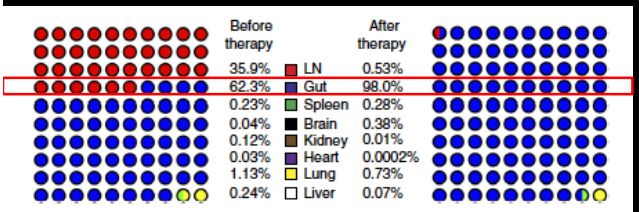


Figure 1 Graphical representation of the proportion of vRNA+ cells in each organ system before and during suppressive ART.

Why does the immune system fail to clear virus from these tissues?

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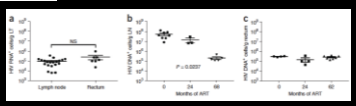


Studies of Mucosal T-cell Function (UCSF Collaboration)

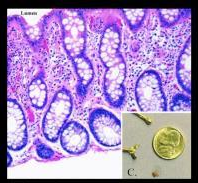
Subject Group	Definition
Early Infection	1-40 wks (median 8)
Controllers (C)	VL <2,000 copies/mL
Viremic (V)	VL >2,000
HAART-treated (Tx)	<75
Seronegative (SN)	<75

Thanks to:

Paired blood and rectal biopsy samples

20 mL blood = 20-40 million PBMC

20-24 biopsies = 5-10 million lymphocytes

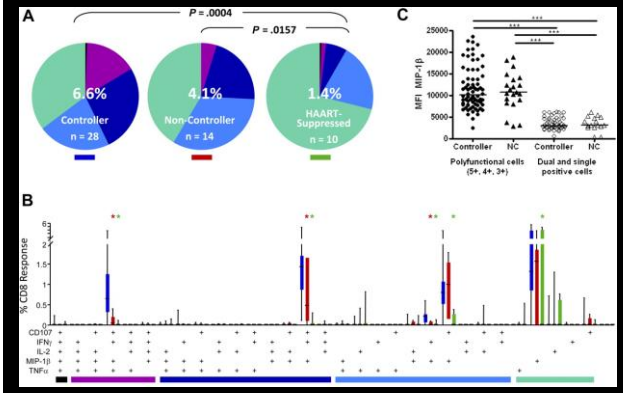


Poles and Anton, JVM 2001

April Ferre, PhD

Mucosal immune responses to HIV-1 in elite controllers: a potential correlate of immune control

April L. Ferre,[1] Peter W. Hunt,[2] J. William Critchfield,[1] Delandy H. Young,[1] Megan M. Morris,[1] Juan C. Garcia,[3] Richard B. Pollard,[4] Hal F. Yee Jr,[5] Jeffrey N. Martin,[6] Steven G. Deeks,[2] and Barbara L. Shacklett[1,4]



Cytokine flow cytometry:

"Polyfunctional*" gut HIV gag-specific CD8+ T-cell responses in Controllers

**MIP-1β, TNFα, IFNγ, IL2, CD107*

**High MIP-1β MFI*

Ferre et al., Blood 2009;113:3978-3989

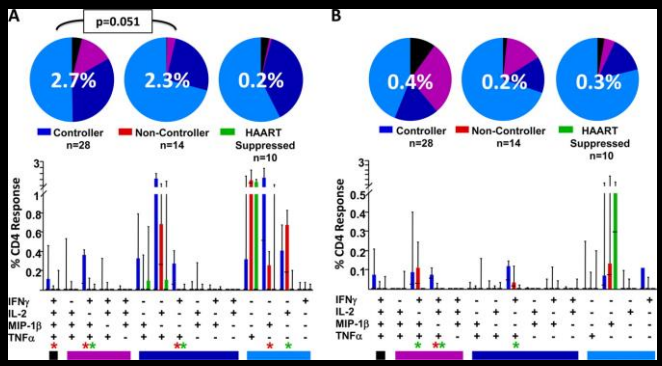
JOURNAL OF VIROLOGY, Nov. 2010, p. 11020–11029 Vol. 84, No. 21
0022-538X/10/$12.00 doi:10.1128/JVI.00980-10

HIV Controllers with HLA-DRB1*13 and HLA-DQB1*06 Alleles Have Strong, Polyfunctional Mucosal CD4+ T-Cell Responses▽†

April L. Ferre,[1] Peter W. Hunt,[4] Delandy H. McConnell,[1] Megan M. Morris,[1] Juan C. Garcia,[2] Richard B. Pollard,[3] Hal F. Yee, Jr.,[5] Jeffrey N. Martin,[6] Steven G. Deeks,[4] and Barbara L. Shacklett[1,3]*

Rectal Mucosa

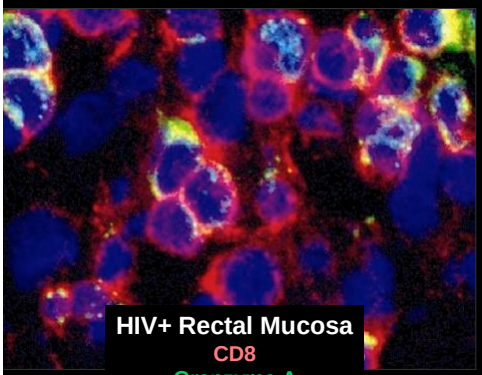
Relative Gut CD4+ T-cell Preservation In Controllers



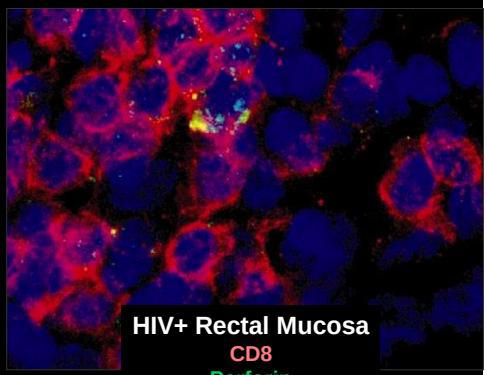
Blood

Ferre et al., J Virol 2010 84:11020-11029

BUT…Surprisingly, CD8+ T-cells from colorectal mucosa contain abundant granzymes, *yet little perforin*



HIV+ Rectal Mucosa
CD8
Granzyme A
Overlay
Nucleic Acid



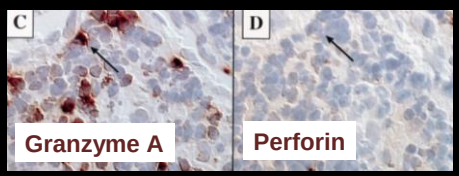
HIV+ Rectal Mucosa
CD8
Perforin
Overlay
Nucleic Acid

Shacklett et al., J. Immunol. 2004

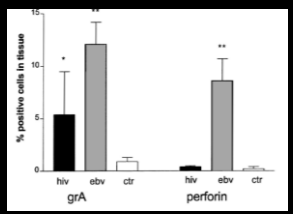
Perforin is not co-expressed with granzyme A within cytotoxic granules in CD8 T lymphocytes present in lymphoid tissue during chronic HIV infection

Jan Andersson[a], Homira Behbahani[a], Judy Lieberman[b], Elizabeth Connick[c], Alan Landay[d], Bruce Patterson[e], Anders Sönnerborg[a], Karin Loré[a], Stefania Uccini[f] and Thomas E. Fehniger[a]

Lymph Nodes



HIV vs EBV (Mononucleosis)



Andersson et al., AIDS 1999

The "Textbook" Description of Cytotoxic T-Lymphocytes (CTL)

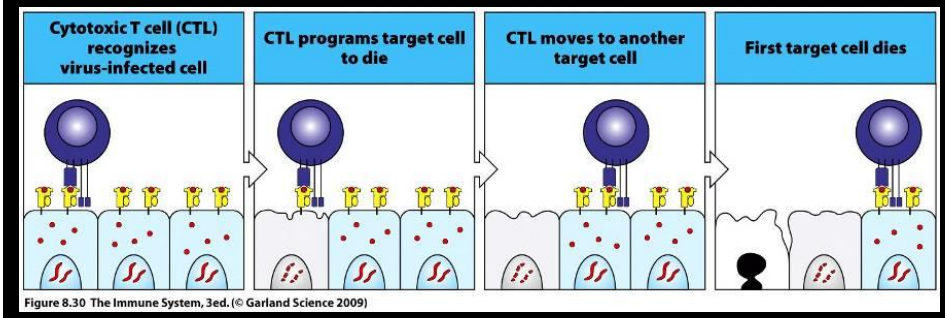


Figure 8.30 The Immune System, 3ed. (© Garland Science 2009)

Parham, Janeway, others…

- "Serial Killers" of infected cells
- Kill via granule exocytosis

Delivery of Cytotoxic Granules: the CTL "Kiss of Death"

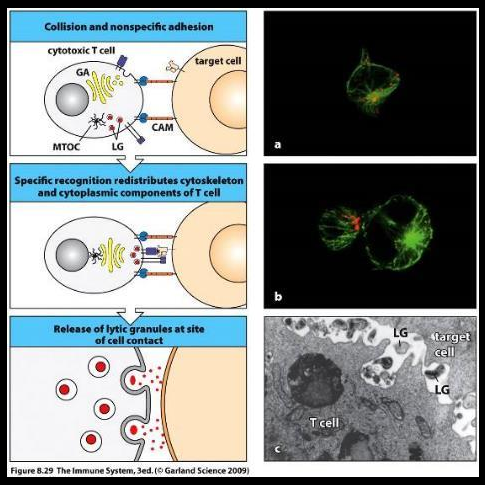
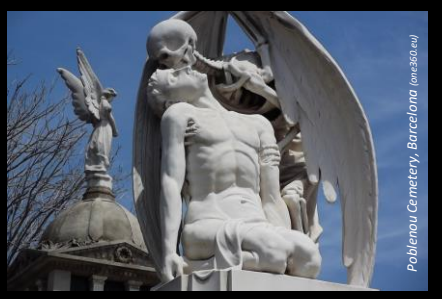


Figure 8.29 The Immune System, 3ed. (© Garland Science 2009)



- Perforin (pore-forming protein)
- Granzymes A, B (serine proteases)
- Caspase activation
- Apoptotic cascade

Hypotheses:

- Perforin expression is tightly regulated (limited) in mucosal and lymphoid tissues
- Expression of cytotoxic effector molecules (perforin and granzymes A, B) is differentially regulated
- HIV-specific CD8+ T-cells in mucosal tissues may be less 'cytotoxic' than their counterparts in blood, and programmed for non-cytolytic effector functions
- This may serve to protect tissue integrity, but may also make pathogen-specific T-cell responses less effective in the gut
  - *Disadvantage vs chronic viral pathogens such as HIV?*
  - *Do HIV Controllers make more perforin than progressors?*

Investigating the Effector Functions of Mucosal CD8+ T-cells

Brenna Kiniry, PhD

Predominance of weakly cytotoxic, T-bet^{Low}Eomes^{Neg} CD8^{+} T-cells in human gastrointestinal mucosa: implications for HIV infection

BE Kiniry[1], A Ganesh[1], JW Critchfield[1], PW Hunt[2], FM Hecht[3], M Somsouk[4], SG Deeks[3] and BL Shacklett[1,5]

VOLUME 10 NUMBER 4 | JULY 2017 | www.nature.com/mi

Differential Expression of CD8^{+} T Cell Cytotoxic Effector Molecules in Blood and Gastrointestinal Mucosa in HIV-1 Infection

Brenna E. Kiniry, Peter W. Hunt, Frederick M. Hecht, Ma Somsouk, Steven G. Deeks and Barbara L. Shacklett

J Immunol published online 19 January 2018
http://www.jimmunol.org/content/early/2018/01/19/jimmunol.1701532

The Journal of Immunology

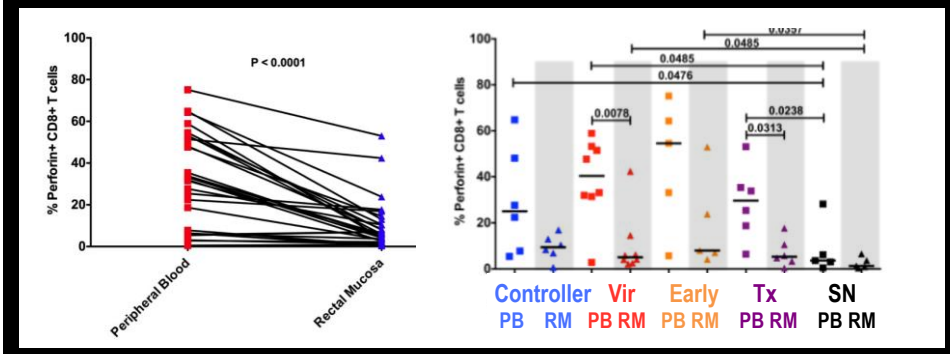
Detection of HIV-1-specific gastrointestinal tissue resident CD8^{+} T-cells in chronic infection

Brenna E Kiniry[1], Shengbin Li[2], Anupama Ganesh[1], Peter W Hunt[3], Ma Somsouk[4], Pamela J Skinner[2], Steven G Deeks[5] and Barbara L Shacklett[1,6]

15 November 2017. doi:10.1038/mi.2017.96

Mucosal Immunology
nature publishing group

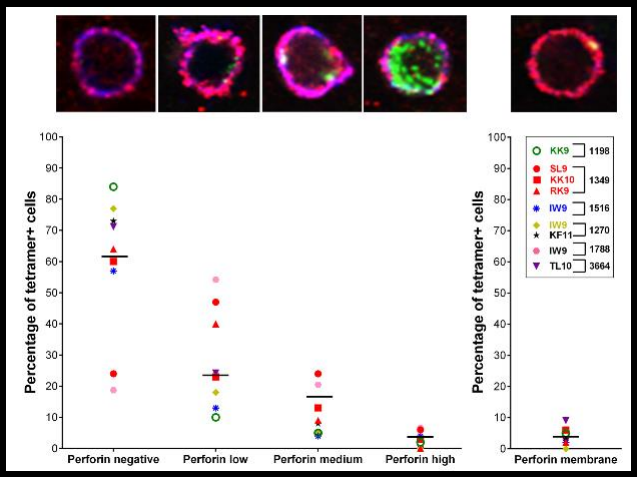
‘Baseline’ perforin expression is significantly lower in mucosal T-cells compared to blood



Gut perforin is highest in early infection and in viremic individuals, lowest in seronegatives; NOT elevated in Controllers

Kiniry et al., Mucosal Immunology 2017, 10(4):1008-1020

Low perforin in mucosal CD8+ T-cells that are HIV-specific



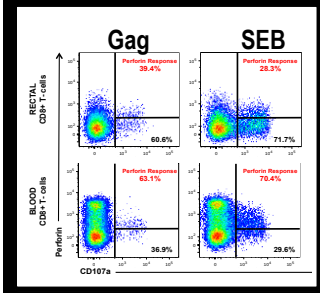
MHC-I tetramers
Perforin
CD8

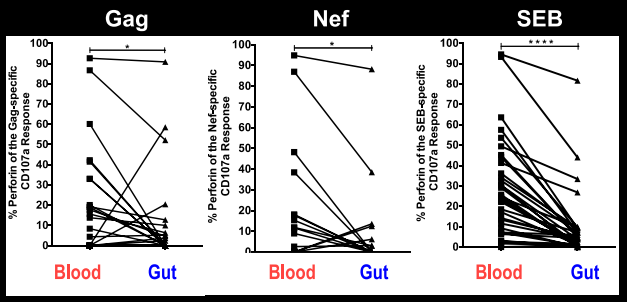
N = 6 HIV controllers

Shengbin Li
Kati Kovacs
Pam Skinner
and our group
In preparation

Perforin is NOT highly expressed in gut CD8+ T-cells of HIV Controllers

In response to TCR stimulation, perforin is more strongly upregulated in blood than gut

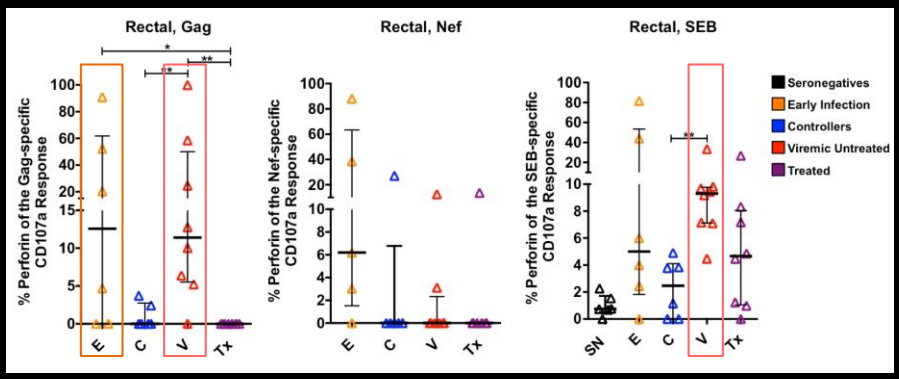




Perforin-expressing cells reported as percentage of degranulating, CD107+ cells

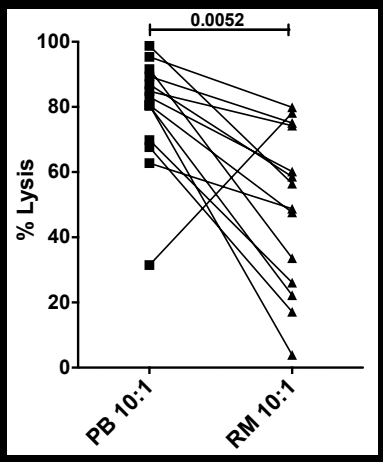
Kiniry et al., Mucosal Immunology 2017, 10(4):1008-1020

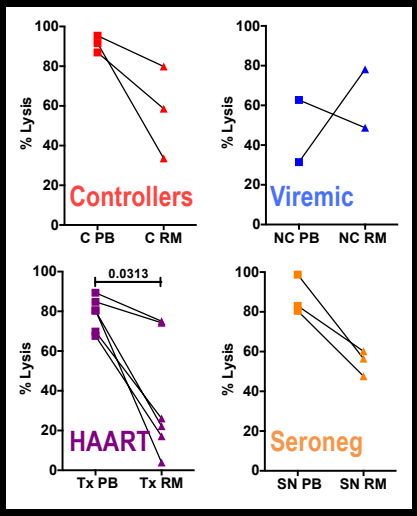
De novo perforin response is stronger in early infection and viremic chronic infection compared to treated infection, and is not elevated in HIV controllers



Kiniry et al., Mucosal Immunology 2017, 10(4):1008-1020

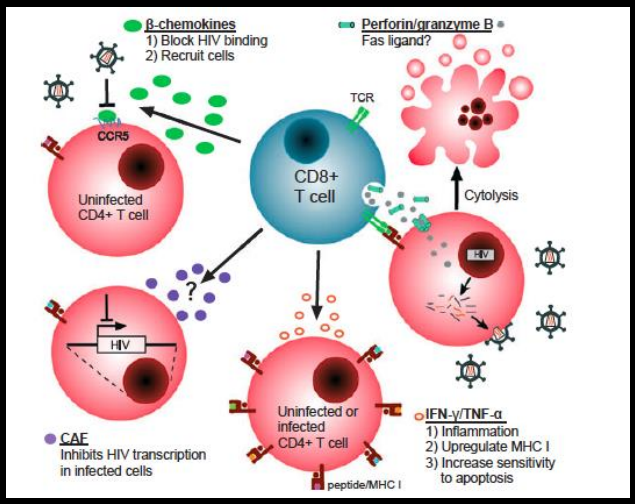
CD8+ T-cell cytotoxic capacity Is significantly lower in rectal mucosa compared to blood





Kiniry et al., Mucosal Immunology 2017, 10(4):1008-1020

... So are colorectal CD8+ T-cells primarily *non-cytolytic* effectors?

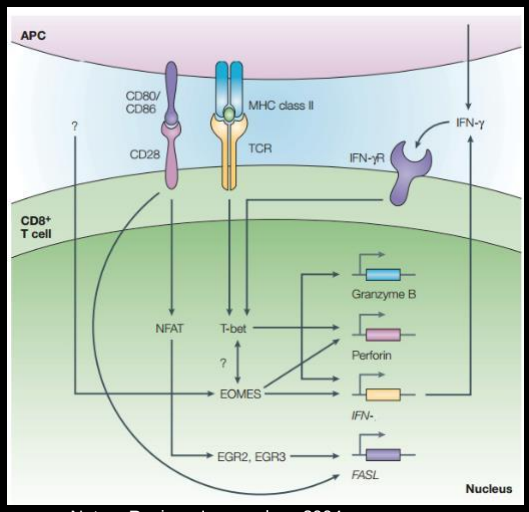


Less cytotoxic capacity…

→ Lower capacity for inflammation?
→ Less able to eradicate viral infection?

How is Cytotoxicity Regulated?

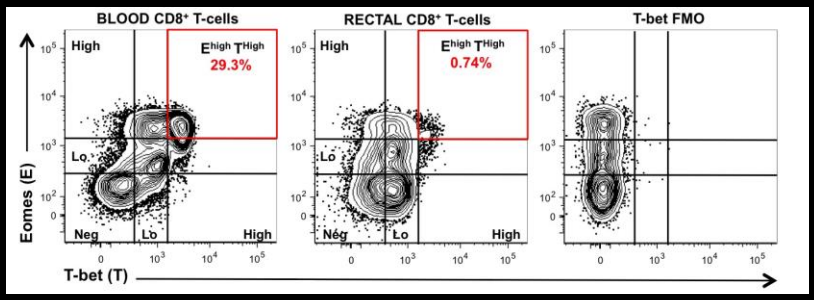
Perforin expression is regulated by transcription factors T-bet and Eomesodermin

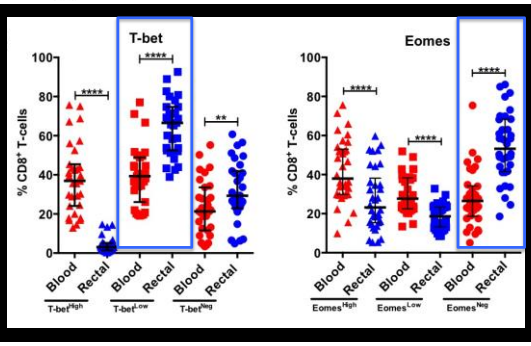


Nature Reviews Immunology 2004 Nov;4(11):900-11.

- **T-bet:** 530-aa protein containing T-box DNA-binding domain
- induced early after TCR stimulation or IFN-γR
- binds to perforin promoter
- synergizes with **EOMESODERMIN (Eomes)**
- ***T-bet deficient NK, CD8⁺ T cells have reduced perforin and cytotoxicity***

Colorectal CD8+ T-cells are mainly T-bet^{low}, Eomes^{neg}





Kiniry et al., Mucosal Immunology 2017 10(4):1008-1020

Article

Cell Reports

HIV-Specific CD8⁺ T Cells Exhibit Reduced and Differentially Regulated Cytolytic Activity in Lymphoid Tissue

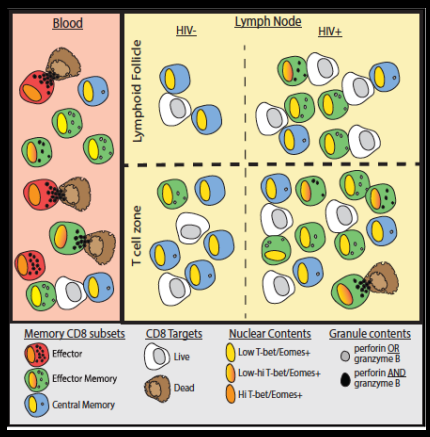
Reuter et al., 2017, Cell Reports 21, 3458–3470
December 19, 2017 © 2017 The Authors.
https://doi.org/10.1016/j.celrep.2017.11.075

Authors
Morgan A. Reuter, Perla M. Del Rio Estrada, Marcus Buggert, ..., David H. Canaday, Gustavo Reyes-Terán, Michael R. Betts

Correspondence
betts@pennmedicine.upenn.edu

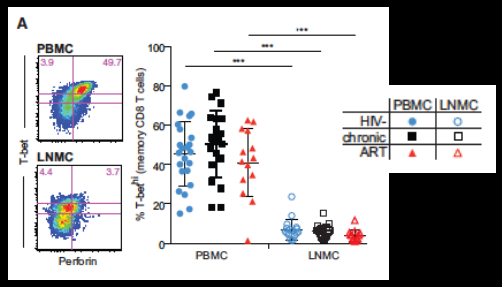
In Brief

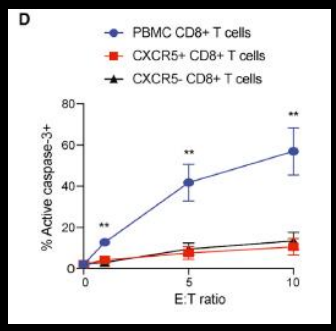
Reuter et al. show that lymphoid tissue CD8⁺ T cells from HIV-infected and uninfected individuals do not possess phenotypic, functional, or transcriptional regulatory properties of cytolytic T cells equivalent to those found in circulation. Their findings suggest that the failure to eliminate HIV could be related to compartmentalized CD8⁺ T cell function favoring noncytolytic responses in lymphoid tissue.

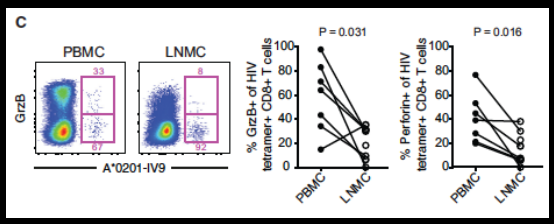


"…the failure to eliminate HIV could be related to compartmentalized CD8+ T-cell function favoring noncytolytic responses in lymphoid tissue"

Lymph Node CD8+ T-cells are also Perforin^{Low}, T-bet^{Low}, and weakly cytotoxic







Reuter et al., 2017, Cell Reports *21*, 3458–3470
December 19, 2017 © 2017 The Authors.
https://doi.org/10.1016/j.celrep.2017.11.075

'Tissue resident' T-cells and HIV infection

Long-lived epithelial immunity by tissue-resident memory T (T_{RM}) cells in the absence of persisting local antigen presentation

Laura K. Mackay, Angus T. Stock, Joel Z. Ma, Claerwen M. Jones, Stephen J. Kent, Scott N. Mueller, William R. Heath, Francis R. Carbone[1], and Thomas Gebhardt[1]

Department of Microbiology and Immunology, University of Melbourne, Melbourne 3010, Australia

PNAS 2012,109:8037

The developmental pathway for CD103+CD8+ tissue-resident memory T cells of skin

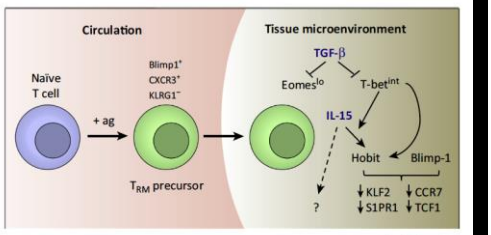
Laura K Mackay[1,5], Azad Rahimpour[1,5], Joel Z Ma[1,5], Nicholas Collins[1], Angus T Stock[1], Ming-Li Hafon[1], Javier Vega-Ramos[1], Pilar Lauzurica[2], Scott N Mueller[1], Tijana Stefanovic[3], David C Tscharke[3], William R Heath[1], Michael Inouye[1,4], Francis R Carbone[1,6] & Thomas Gebh

Nat Immunol 2013, 14:1294

T-box Transcription Factors Combine with the Cytokines TGF-β and IL-15 to Control Tissue-Resident Memory T Cell Fate

Laura K. Mackay,[1,*] Erica Wynne-Jones,[1] David Freestone,[1] Daniel G. Pellicci,[1,2] Lisa A. el Kallies,[3] Gabrielle T. Belz,[3,8] and Francis R. The University of Melbourne, The Peter Doherty Institu

Immunity 2015, 43:1101



IMMUNOLOGY

Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes

Laura K. Mackay,[1,2,†] Martina Minnich,[3] Natasja A. M. Kragten,[4] Yang Liao,[5,6] Benjamin Nota,[7] Cyril Seillet,[1,8] Ali Zaid,[1] Kevin Man,[1,8] Simon Preston,[1,8] David Freestone,[1] Asolina Braun,[1] Erica Wynne-Jones,[1] Felix M. Behr,[4,9,10] Regina Stark,[4] Daniel G. Pellicci,[1,2] Dale I. Godfrey,[1,2] Gabrielle T. Belz,[1,8] Marc Pellegrini,[1,8] Shi,[5,6] Francis R. Carbone,[1] P. J. M. van Gisbergen[4,9,10,6,*,‡]

Science 2016, 352:460

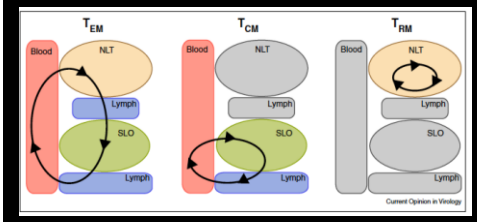
Available online at www.sciencedirect.com

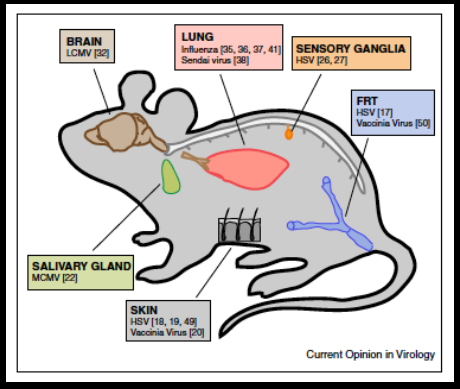
ScienceDirect

Current Opinion in Virology

Tissue resident memory T cells and viral immunity

Pamela C Rosato[1,2], Lalit K Beura[1,2] and David Masopust[1,2]

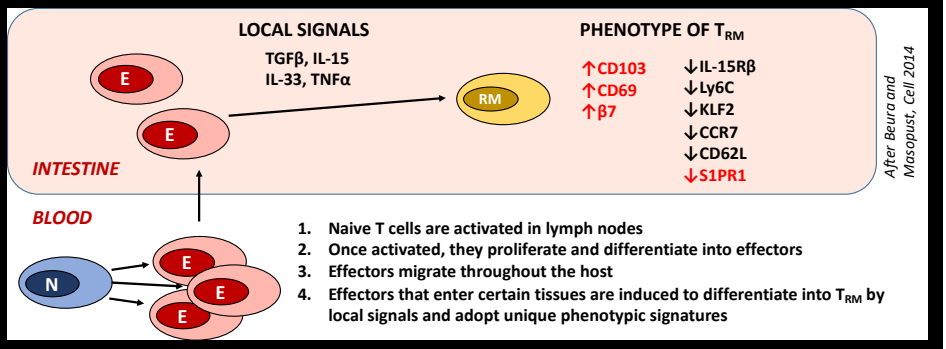




- T_{RM} are non-recirculating memory cells
- Distinct from T_{EM}, T_{CM}

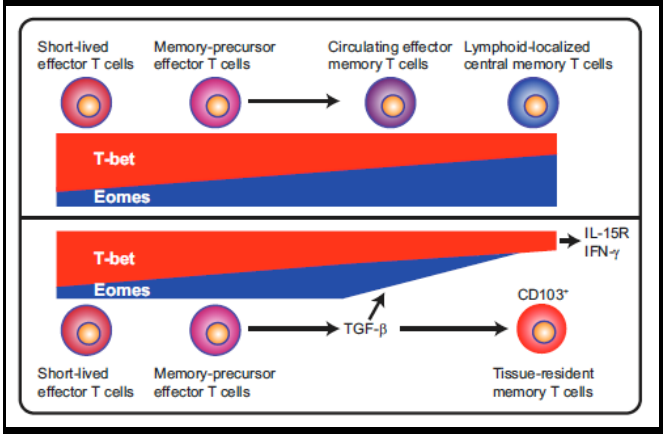
Current Opinion in Virology 2017, 22:44–50

The tissue milieu induces T_{RM} differentiation and maintenance



Beura and Masopust, Cell 2014

T_{RM} development involves loss of Eomes, downregulation of T-bet

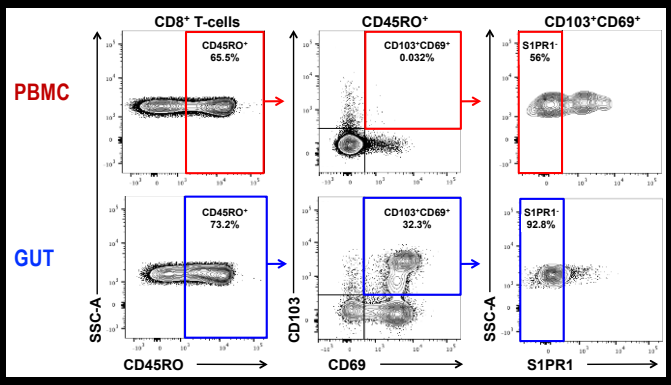


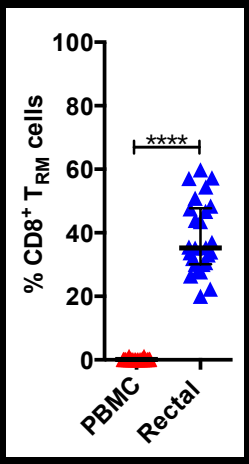
- Driven by TGF-β
- Residual T-bet is important for T_{RM}

T_{RM} phenotype is linked to tissue localization and NOT to TCR specificity

Mackay, Carbone,et al., 2015

CD8+ T-cells with a 'tissue resident' phenotype are abundant in human colorectal mucosa

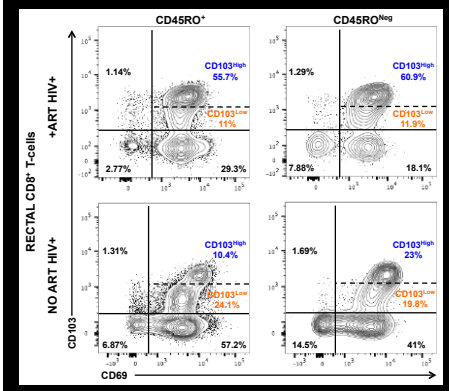


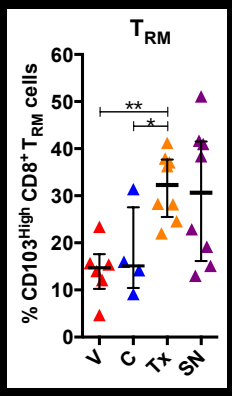


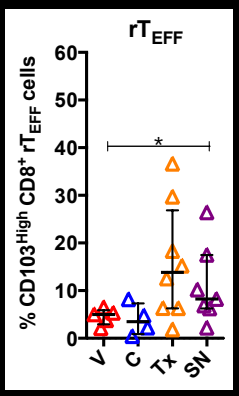
CD103+, CD69+, S1PR1^{neg}

Kiniry et al., Mucosal Immunology 2018, 11:909

Two major "resident" subsets:
T_{RM} (CD45RO$^+$, memory) and rT_{EFF} (CD45RO$^-$, effector)



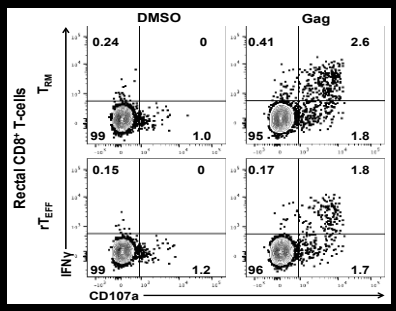


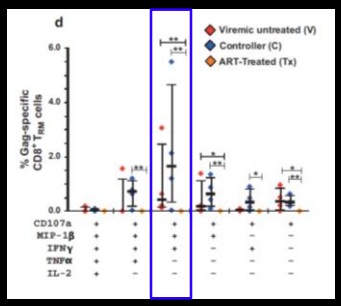


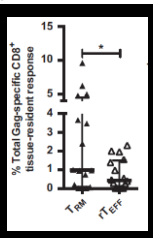
Viremic (V)
Controller (C)
On HAART (Tx)
Seronegative (SN)

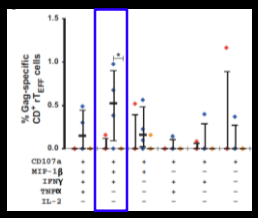
Kiniry et al., Mucosal Immunology 2018, 11:909

HIV Controllers have strong Gag-specific CD8+ T_{RM} and rT_{EFF} responses







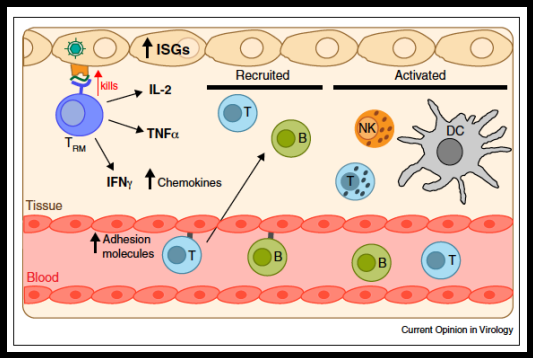


Kiniry et al., Mucosal Immunology 2018, 11:909

Tissue Resident CD8+ T-Cells: *Beyond Cytotoxicity*

- **β-Chemokine production**
  - Ferre et al., Blood 2009: Polyfunctional HIVgag-specific T-cells produce MIP-1β
- **IFNγ production**
  - Masopust et al., T_{RM} cell IFNγ indirectly promotes recruitment of T and B cells to tissue infection sites
- **Continuous patrol of tissues**
  - Ariotti et al., PNAS 2012: HSV-gB-specific mouse CD8 cells patrol skin after resolution of infection, along with LCs
- **Triggering of innate-like responses: "neighborhood watch"**
  - Ariotti et al., Science 2014: recruitment of other cell types

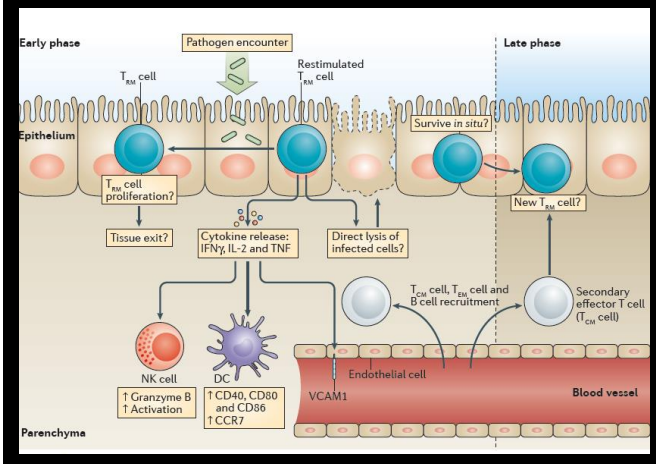
T_{RM} Trigger a Cascade of Immunostimulatory and Antiviral Responses in Tissues



Current Opinion in Virology 2017, 22:44-50

- ***Some express high granzyme B, but many are perforin low***
- ***Upon TCR stimulation, many secrete TNFα, IFNγ, IL2***
- ***Leads to upregulation of CXCL9, CXCL10, and of VCAM-1 on endothelial cells***
- ***Recruitment of memory CD8+ T-cells, CD4+ T-cells, B-cells***
- ***IFN stimulated genes (ISGs) are upregulated in surrounding cells***
- ***Activation of other cell types: NK, DC, bystander CD8+ T-cells***

The T_{RM} "neighborhood watch"



- *Immediate triggering*
- *Cytokine release*
- *Recruitment and activation of other cell types; upregulation of adhesion molecules*

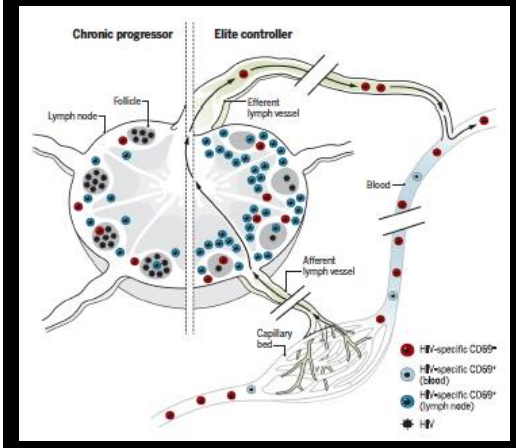
Mueller SN and Mackay LN, NRI Feb 2016

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



Lymph Nodes:

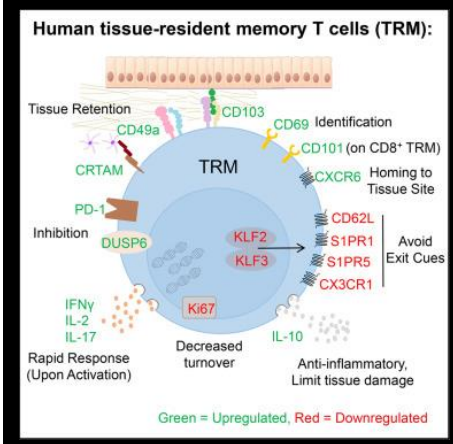
- **HIV-specific T_{RM} (CD69$^+$) are more abundant and more cytolytic than non-T_{RM}**
- **HIV-specific T_{RM} are more abundant in Controllers than Progressors**

Buggert, Betts et al., Science Immunology 2018
Reviewed in Roan, Sci Imm 2018

Article

Cell Reports

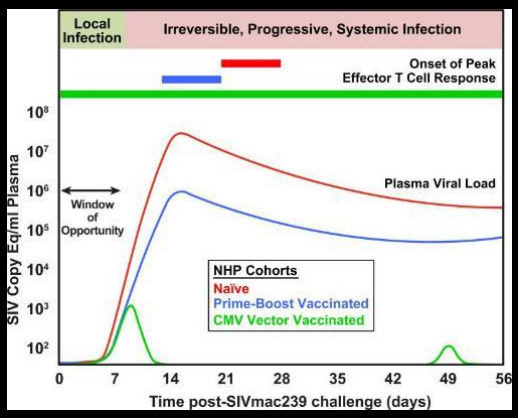
Human Tissue-Resident Memory T Cells Are Defined by Core Transcriptional and Functional Signatures in Lymphoid and Mucosal Sites



- **T_{RM} populate multiple tissues**
- **T_{RM} have unique adhesion and migratory abilities and functional capacities**
- **A core signature defining human T_{RM} is enriched within CD69+ memory T-cells**
- ***Kumar, Farber et al., Cell Reports 2017, 20:2921-2934***

Farber lab (Columbia Univ, NY, USA)

Can we utilize T_{RM} to prevent and/or cure mucosal infections?



New Paradigms for HIV/AIDS Vaccine Development

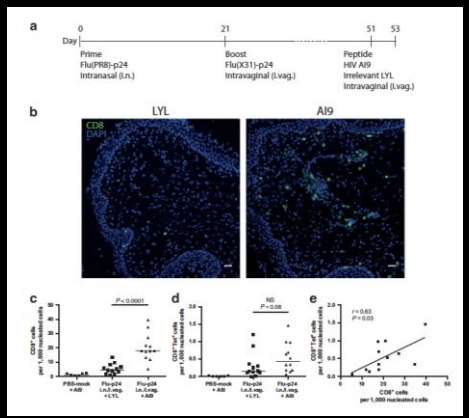
Louis J. Picker,[1] Scott G. Hansen,[1] and Jeffrey D. Lifson[2]

Picker et al., Annu. Rev. Med. 2012 63:95-111

T-cell based vaccines have demonstrated impressive protection in the SIVmac model

Induction of vaginal-resident HIV-specific CD8 T cells with mucosal prime–boost immunization

H-X Tan[1], AK Wheatley[1,2], R Esterbauer[1], S Jegaskanda[1], JJ Glass[1,2], D Masopust[3,4], R De Rose[1,5,7] and SJ Kent[1,2,6,7]



- **Murine model**
- **Combined intranasal and intravaginal immunization**
- **Recombinant HIV-flu vectors**

Induced HIV-specific CD8+ T-cells in vaginal epithelium

Upon TCR stimulation → recruitment of adaptive and innate effectors:

CD8+, CD4+, B, NK

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How can we harness T_{RM} to help prevent and/or cure mucosal infections?

- **Mucosal vaccination**
  - ***Vaccines based on mucosal organisms***
  - ***Delivery via mucosal routes***
  - ***Use of mucosal adjuvants***
  - ***Use of replicating vectors***
  - ***Induction of mucosal homing pathways***
- **Will require better understanding of T_{RM} heterogeneity, induction, maintenance, and functionality**

Summary: T_{RM} cells in HIV infection

- **Human gastrointestinal mucosa is enriched for CD8+ T-cells with a $T_{resident}$ phenotype;**
- **Our "classical" understanding of tissue T-cell subsets as recirculating populations is being replaced by a more nuanced view that accounts for tissue residency;**
- **HIV Controllers have strong, polyfunctional T_{RM} responses, suggesting a role in fighting chronic infection;**
- **It may be possible to capitalize on specific features of T_{RM} , including their ability to recruit innate and adaptive effectors, to improve vaccines and immunotherapies.**

Acknowledgments

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- *ZSFGH/UCSF Clinical Staff*
- *Study Volunteers*