Trafficking of HIV out of the mucosa

Role for Integrin α₄β₇⁺ in HIV and SIV Transmission and Pathogenesis?

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Integrin $\alpha_4\beta_7$: gut homing receptor

- $\alpha_4\beta_7$ is a cell-surface receptor expressed on T, B and NK cells. (GALT)
- $\alpha_4\beta_7$ binds to MAdCAM, an adhesion receptor that is primarily expressed on HEV’s in GALT.
- The tissue-specific expression of MAdCAM in GALT defines $\alpha_4\beta_7$ as the gut-homing receptor
Susceptibility of the GI Tract to SIV and HIV

Gastrointestinal Tract as a Major Site of CD4+ T Cell Depletion and Viral Replication in SIV Infection

Ronald S. Veazey, MaryAnn DeMaria, Laura V. Chalifoux, Diniel E. Shvetz, Douglas R. Pauley, Heather L. Knight, Michael Rosenzweig, R. Paul Johnson, Ronald C. Desrosiers, Andrew A. Lackner*

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Severe CD4+ T-Cell Depletion in Gut Lymphoid Tissue during Primary Human Immunodeficiency Virus Type 1 Infection and Substantial Delay in Restoration following Highly Active Antiretroviral Therapy

Moraima Guadalupe,1 Elizabeth Reay,1 Sumathi Sankaran,1 Thomas Prindiville,2 Jason Flamm,3 Andrew McNeil,2,4 and Satya Dandekar1,2*

Primary HIV-1 Infection Is Associated with Preferential Depletion of CD4+ T Lymphocytes from Effector Sites in the Gastrointestinal Tract

Saurabh Mehandru,1 Michael A. Poles,1,2 Klara Tenner-Racz,3 Amir Horowitz,1,2 Arlene Hurley,1 Christine Hogan,1 Daniel Boden,1 Paul Racz,2 and Martin Markowitz1

Nature Reviews | Immunology
Persistent Depletion of CD4+ T cells in the GI Tract Despite Normalization in the Peripheral Blood in ART-treated Patients

Primary HIV-1 Infection Is Associated with Preferential Depletion of CD4+ T Lymphocytes from Effector Sites in the Gastrointestinal Tract

Saurabh Mehandru,1 Michael A. Poles,1,2 Klara Tenner-Racz,3
Amir Horowitz,1,2 Arlene Hurley,1 Christine Hogan,1 Daniel Boden,1
Paul Racz,3 and Martin Markowitz1

Mechanisms of Gastrointestinal CD4+ T-Cell Depletion during Acute and Early Human Immunodeficiency Virus Type 1 Infection

Saurabh Mehandru,1 Michael A. Poles,1,2 Klara Tenner-Racz,3 Victoria Manuelli,1 Patrick Jean-Pierre,1
Peter Lopez,1 Anita Shet,1 Andrea Low,1 Hiroshi Mohri,1 Daniel Boden,1
Paul Racz,3 and Martin Markowitz1

Lack of Mucosal Immune Reconstitution during Prolonged Treatment of Acute and Early HIV-1 Infection

Saurabh Mehandru1, Michael A. Poles1,2, Klara Tenner-Racz3, Patrick Jean-Pierre1, Victoria Manuelli1, Peter Lopez1,
Anita Shet1, Andrea Low1, Hiroshi Mohri1, Daniel Boden1, Paul Racz3, Martin Markowitz1

- CD4+ T cells are preferentially depleted in the GI tract in acute and early HIV-1 infection
- GI CD4+ T cells harbor a higher viral burden compared to peripheral blood CD4+ T cells
- Lack of reconstitution in GI derived CD4+ T cells during prolonged ART in the majority of patients
The GI tract is preferentially targeted during acute/early HIV-1 and SIV infections with consequent damage to the gut.

In the majority of patients, long term antiretroviral therapy does not efficiently reconstitute mucosal CD4\(^+\) T cells.
Integrin $\alpha_4\beta_7$ and HIV Infection

HIV-1 envelope protein binds to and signals through integrin $\alpha_4\beta_7$, the gut mucosal homing receptor for peripheral T cells

*Integrin $\alpha_4\beta_7$ is the gut-homing receptor*
The integrin $\alpha_4\beta_7$ forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1

Claudia Cicala$^{a,1,2}$, Elena Martinelli$^{a,1}$, Johnathan P. McNally$^a$, Diana J. Goode$^a$, Ravindra Gopaul$^a$, Joseph Hiatt$^a$, Katija Jelicic$^c$, Shyamasundaran Kottillil$^b$, Katilyn Macleod$^b$, Angeline O'Shea$^a$, Nikita Patel$^b$, Donald Van Ryk$^a$, Danian Wei$^a$, Massimiliano Pascuccio$^a$, Ling Yi$^b$, Lyle McKinnon$^c$, Preson Izulla$^d$, Joshua Kimani$^d$, Rupert Kauf$^c$, Anthony S. Fauci$^{a,2}$, and James Arthos$^a$

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$\alpha_4\beta_7^+/CD4^+$ T cells define a subset of CD4$^+$ T cells that are metabolically active, Ki67$^+$, CCR5$^{\text{high}}$, CXCR4$^{\text{low}}$.

$\alpha_4\beta_7^+/CD4^+$ T cells appear in the gut and genital mucosa.
Memory Peripheral and Genital Tract $\alpha_4\beta_7^{\text{high}}$ Memory CD4$^+$ T Cells Express CCR5 and are Highly Activated.

- Naïve CD4$^+$ T cells express an intermediate level of $\alpha_4\beta_7$
- Memory CD4$^+$ T cells express high levels of $\alpha_4\beta_7$: $\alpha_4\beta_7^{\text{high}}$ CD4$^+$ T cells
Preferential depletion of $\alpha_4\beta_7^{\text{high}}$ CD4$^+$ T cells

- intracellular p24 staining of CD4$^+$ T cells, day 3, 6, 8, post infection
Result: the overall rate of HIV transmission observed in these discordant couples: 0.0012/coital act.
Potential barriers to HIV transmission across the genital mucosa.

“The entering viruses must interact with susceptible CD4+ CCR5+ T cells to propagate since entry into non permissive resting CD4+ T cells will result in nonproductive infection”

Monaco et al. (2017) Current Topics in Microbiology and Immunology
$\alpha_4\beta_7^+/\text{CD}4^+$ T Cells are a Prime Target for Productive Infection in Mucosal Tissues
Characterization of a Human Cervical CD4⁺ T Cell Subset Coexpressing Multiple Markers of HIV Susceptibility

Lyle R. McKinnon,*,† Billy Nyanga,†,1 Duncan Chege,*,† Preston Izulla,† Makobu Kimani,† Sanja Huibner,⁎ Lawrence Gelmon,†,‡ Katharine E. Block,§ Claudia Cicala,§ A. Omu Anzala,†,§ James Arthos,§ Joshua Kimani,†,‡ and Rupert Kaul⁎,†,II

The Journal of Immunology, 2011, 187

Cervical Cytobrush CD4 T Cells:
Th17 Cells Are Preferentially Infected Very Early after Vaginal Transmission of SIV in Macaques

Daniel J. Stieh, Edgar Matias, Huanbin Xu, Angela J. Fought, James L. Blanchard, Preston A. Marx, Ronald S. Veazey, Thomas J. Hope
HSV2 Infection upregulates $\alpha_4\beta_7$ on the surface of $CD4^+ T$ cells.

Martinelli et al. PLoS Path 2011


Epithelium

HSV-2

HIV-1

CXCL10? IL7?

$\alpha_4\beta_7$?

RA?

R>1

% $\alpha_4\beta_7^+/CD4^+ T$ cells

p < 0.001

HSV-2 Negative

HSV-2 Positive

Gut and MLN

0 10 20 30 40

0

**α₄β⁷⁺/CD4⁺ T Cells are Targeted in Acute Infection**

- Blood. 2001 Nov;98(10): 3169-3171
  - Preferential and persistent depletion of CCR5⁺ T-helper lymphocytes with nonlymphoid homing potential despite early treatment of primary infection

  - Monitoring α₄β₇ integrin expression on circulating CD4⁺ T cells as a surrogate marker for tracking intestinal CD4⁺ T-cell loss in SIV infection
    - X Wang, H Xu, AF Gill, B Pahar, D Kempf, T Rasmussen, AA Lackner and RS Veazey.

  - Blocking of Gut-Homing Integrin during Acute Infection Leads to Decreased Plasma and Gastrointestinal Tissue Viral Loads in Simian Immunodeficiency Virus-Infected Rhesus Macaques
The Frequency of $\alpha_4\beta_7^{\text{hi}}$ Memory CD4$^+$ T Cells is Directly Correlated with Risk of Acquisition in Rhesus Macaques

The Frequency of $\alpha_4\beta_7^{\text{hi}}$ Memory CD4$^+$ T Cells Correlates With Susceptibility to Rectal Simian Immunodeficiency Virus Infection

Elena Martinelli, PhD, MPH,* Filippo Veglia, PhD,* Diana Goode, PhD,* Natalia Guerra-Perez, PhD,* Meropi Aravantinou, MS,* James Arthos, PhD,‡ Michael Piatak, Jr., DMV, PhD,‡ Jeffery D. Lifson, MD,‡ James Blanchard, PhD,§ Agegnehu Gettie, BS,II and Melissa Robbiani, PhD*

J Acquir Immune Defic Syndr. 2013

Adjuvant-dependent innate and adaptive immune signatures of risk of SIVmac251 acquisition

Monica Vaccari¹,², Shari N Gordon¹,², Slim Fourati²,², Luca Schifanella¹,³,², Namal P M Livanage¹,²,³ & Genoveffa Franchini¹,²,³

2016 NATURE MEDICINE

R = −0.62
P = 0.02

Number of IR Challenges

% of $\alpha_4\beta_7^{\text{hi}}$ Ki67$^+$ CD4$^+$ T Cells
Effect of ART on Colonic $\alpha_4\beta_7^{hi}$ CD4$^+$ T Cells

- Colonic $\alpha_4\beta_7^{hi}$ CD4$^+$ T cells are not replenished, even after ART treatment initiated in Fiebig I

**CCR5$^+$ CD4$^+$ T cells**

**$\alpha_4\beta_7^{hi}$ CD4$^+$ T cells**
Can Targeting $\alpha_4\beta_7^+$ T cells have an effect on SIV/HIV transmission?
Rhesus Anti-$\alpha_4\beta_7$ Antibody (mAb Act1)

Vedolizumab as Induction and Maintenance Therapy for Crohn's Disease

William J. Sandborn, M.D., Brian G. Feagan, M.D., Paul Rutgeerts, M.D., Ph.D., Stephen Hanauer, M.D., Jean-Frédéric Colombel, M.D., Bruce E. Sands, M.D., Milan Lukas, M.D., Ph.D., Richard N. Fedorak, M.D., Scott Lee, M.D., Brian Bressler, M.D., Irving Fox, M.D., Maria Rosario, Ph.D., Serap Sankoh, Ph.D., Jing Xu, Ph.D., Kristin Stephens, B.A., Catherine Milch, M.D., and Asit Parikh, M.D., Ph.D., for the GEMINI 2 Study Group

Vedolizumab is a human analogue of mAb Act1
Targeting $\alpha_4\beta_7$ integrin reduces mucosal transmission of simian immunodeficiency virus and protects gut-associated lymphoid tissue from infection

Siddappa N Byrareddy$^{1,8}$, Brianne Kallam$^{1,8}$, James Arthos$^2$, Claudia Cicala$^2$, Fatima Nawaz$^2$, Joseph Hiatt$^2$, Ellen N Kersh$^3$, Janet M McNicholl$^3$, Debra Hanson$^3$, Keith A Reimann$^4$, Markus Brameier$^5$, Lutz Walter$^5$, Kenneth Rogers$^6$, Ann E Mayne$^7$, Paul Dunbar$^1$, Tara Villinger$^1$, Dawn Little$^1$, Tristram G Parslow$^1$, Philip J Santangelo$^7$, Francois Villinger$^{1,6}$, Anthony S Fauci$^2$ & Aftab A Ansari$^1
Low-Dose Challenge Study Design

- **α4α7 mAb monkeys**
- **Viral challenges**
- **Intravaginal infection**
- **mAb monkeys**
- **control mAb monkeys**
\( \alpha_4\beta_7 \) mAb prevents and delays mucosal transmission of SIV

**Significant protection**

**Pronounced delay in viremia**

\[ P = 0.002 \]
Individual Plasma Viremia Levels in NHPs Receiving Either $\alpha_4\beta_7$ mAb or Control IgG During Mucosal Challenge with SIVmac251

**Anti-$\alpha_4\beta_7^+$ Antibody**

- 6/12 infected

**IgG Control**

- 10/12 infected

Log plasma viral RNA (copies/ml)

Time after Initial SIVmac251 Challenge (weeks)
**α₄β₇ mAb Protects the Gut**

**pro-viral DNA levels in gastro-intestinal tissue biopsies**

**anti- α₄β₇ treated/infected**

**IgG treated/infected**

<table>
<thead>
<tr>
<th>RCw11</th>
<th>RDg11</th>
<th>Rlz12</th>
<th>ROC11</th>
<th>RRc9</th>
<th>RQm11</th>
</tr>
</thead>
</table>

| RBs9 | REo8 | RHy12 | RHy12 | RKs11 | RLc12 | RRn11 | RVw10 | RWt9 | RZz10 | RCd12 |

- Viral DNA (copies/ng)
- Time after initial SIVmac251 challenge (weeks)
Anti $\alpha_4\beta_7$ Minimizes Loss of Both Memory and Naïve CD4$^+$ T Cells in the Peripheral Blood

![Graphs comparing CD4+ T cell counts across different conditions](image-url)

- **CD4$^+$ T cells (total)**
  - P < 0.001

- **Naïve CD4$^+$ T cells**
  - P = 0.04

- **Central memory CD4$^+$ T cells**
  - P = 0.58

- **Effector memory CD4$^+$ T cells**
  - P = 0.02

Legend:
- Uninfected RMs
- Anti-$\alpha_4\beta_7$ treated infected RMs
- IgG treated infected RMs
Protection of gut tissue preserves peripheral CD4⁺ T cells
Immuno-PET/CT Interrogation of SIV Infected Macaques

Probes

1. $^{64}$Cu-labeled anti-CD4 F(ab’)$_2$

2. $^{64}$Cu-labeled anti-gp120
Immuno-PET/CT Interrogation of SIV Infected Macaques

$\alpha_4\beta_7$ mAb treatment reduced virus in gut, but also in other lymphoid tissues.

Probe $^{64}$Cu-labeled anti-gp120
3 weeks post-infection
Conclusions

1. A primatized monoclonal IgG antibody directed against $\alpha_4\beta_7$ reduces the efficiency of vaginal transmission of SIV in rhesus macaques.

2. $\alpha_4\beta_7^{\text{high}}$CD4$^+$ T cells that reside in, or traffic to, the gut-associated lymphoid tissues (GALT) play a key role in SIV transmission.

3. In macaques that do become infected in the presence of an $\alpha_4\beta_7$ mAb, viremia is delayed and GALT is protected in a significant way.
Can Targeting $\alpha_4\beta_7$ T cells change the pathogenesis of SIV/HIV infection?
Sustained virologic control in SIV+ macaques after antiretroviral and a4b7 antibody therapy

Siddappa N. Byrareddy,1,4† James Arthos,2* Claudia Cicala,2* Francois Villinger,1,3‡ Kristina T. Ortiz,1 Dawn Little,1 Neil Sidell,1 Maureen A. Kane,5 Jianshi Yu,1 Jace W. Jones,5 Philip J. Santangelo,6 Chiara Zurla,6 Lyle R. McKinnon,7§ Kelly B. Arnold,6 Caroline E. Woody,6 Lutz Walter,6 Christian Roos,6 Angela Noll,9 Donald Van Ryk,2 Katija Jelicic,2 Raffaele Cimbro,10 Sanjeev Gumber,3 Michelle D. Reid,1 Volkan Adsay,1 Praveen K. Amancha,3 Ann E. Mayne,1 Tristram G. Parslow,1 Anthony S. Fauci,2 Aftab A. Ansari1†
Schema of Experimental Design ART + anti-α₄β₇ Study

**anti-α₄β₇ Treated Group (n=11)**
- ART administered daily for 3 months
- SIVmac239 (200TCID₅₀)
- ART administration terminated
- Baseline collections
- PMPA (20mg/kg/day) SubQ
- FTC (50mg/kg/day) SubQ
- Integrase inhibitor L-870812 (100mg/kg/day) Oral

**anti-IgG Treated Group (n=7)**
- ART
- α₄β₇ or IgG (50mg/kg)
- Administration initiated
- α₄β₇ or IgG Administration terminated
Combining ART with anti-\(\alpha_4\beta_7\) Promotes Virologic Control in SIV Infected Macaques

SIVmac239 (200TCID\(_{50}\))

ART administered daily for 3 months

PMPA (20mg/kg/day) SubQ
FTC (50mg/kg/day) SubQ
Integrase inhibitor L-870812 (100mg/kg/day) Oral

Baseline collections

Log Plasma Viral RNA (copies/ml)

(Geometric mean)

Pro-viral DNA levels in gastro-intestinal tissue biopsies (copies/ng DNA)

Weeks

Phase I Phase II Phase III Phase IV Phase V

**ART****

\(\alpha_4\beta_7\)

\(\text{IgG}\)

\(\alpha_4\beta_7\) or IgG

Phase I Phase II Phase III Phase IV Phase V

**ART****
ART + anti-α₄β₇ Treatment Promotes the Restoration of CD4⁺ T Cells in SIV Infected Macaques

Blood CD4 counts indicate absolute cell numbers.
Gut CD4 frequencies based on gated population of CD45⁺ cells.
ART + $\alpha_4\beta_7$ mAb Promotes Durable Preservation of CD4$^+$ cells in Gut and Lymphoid Tissues

CD4$^+$ Cell PET imaging of animals treated with combination ART + $\alpha_4\beta_7$ mAb

**week 50**

Rld14 ($\alpha_4\beta_7$ mAb)  Rlt11 (IgG)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>SUVmax Rld14</th>
<th>SUVmax Rlt11</th>
</tr>
</thead>
<tbody>
<tr>
<td>NALT and Facial Cranial LN</td>
<td>0.50</td>
<td>0.10</td>
</tr>
<tr>
<td>Axillary LN</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Gastrointestinal Tract</td>
<td>1.50</td>
<td>0.20</td>
</tr>
<tr>
<td>Inguinal LN</td>
<td>2.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

**SUVmean**

- Spleen: $\alpha_4\beta_7$ mAb > IgG
- Muscle: $\alpha_4\beta_7$ mAb < IgG
Durable Control of Viremia and Durable Preservation of CD4 cells in ART + $\alpha_4\beta_7$ mAb treated Macaques

**Log plasma viral RNA (copies/ml)**

- **ART + IgG**
- **ART + $\alpha_4\beta_7$ mAb**

**Immuno-histological evaluation of CD4+ T cells in GIT biopsy specimens**
Combination ART + $\alpha_4\beta_7$ mAb reduces gut viral load over ART alone during the dual-therapy (aviremic) phase.

SIV gp120 PET imaging
$\alpha_4\beta_7^+/\text{CD}4^+ \text{ T Cells are a Prime Target for Productive Infection in Mucosal Tissues}$
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