Nonhuman primate studies for the prevention of HIV infection

Studies of mucosal pathogenesis, transmission, and prevention in the macaque model of HIV

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Clinic, Lab, Office, and Administration

Breeding colony

Room for expansion
<table>
<thead>
<tr>
<th>HIV-1 in humans</th>
<th>SIV in macaques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lentivirus</td>
<td>Lentivirus</td>
</tr>
<tr>
<td>Gag, Pol, Env, Rev, Tat, Vpr, Vpu</td>
<td>Gag, Pol, Env, Rev, Tat, Vpr, Vpx</td>
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<tr>
<td>CD4/CK Rc tropism</td>
<td>CD4/CK Rc tropism</td>
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<tr>
<td>CCR5 tropic (most)</td>
<td>CCR5 tropic (most)</td>
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<tr>
<td>Asymptomatic stage (~10 yrs)</td>
<td>Asymptomatic stage (~1-3 yrs)</td>
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<tr>
<td>CD4+ T cell decline</td>
<td>CD4+ T cell decline</td>
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<tr>
<td>Initial viral peak followed by low levels</td>
<td>Initial viral peak followed by low levels</td>
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<tr>
<td>Persistent infection</td>
<td>Persistent infection</td>
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<tr>
<td>Wasting disease, diarrhea</td>
<td>Wasting disease, diarrhea</td>
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<tr>
<td>Lymphadenopathy</td>
<td>Lymphadenopathy</td>
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<tr>
<td>Massive intestinal CD4 depletion in acute infection</td>
<td>Massive intestinal CD4 depletion in acute infection</td>
</tr>
<tr>
<td>“Neoplasia”</td>
<td>“Neoplasia”</td>
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<tr>
<td>Lymphoma</td>
<td>Lymphoma</td>
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<tr>
<td>Kaposi’s sarcoma (HHV-8)</td>
<td>RRV infection (HHV-8 like)</td>
</tr>
<tr>
<td>AIDS (opportunistic infections)</td>
<td>AIDS (opportunistic infections)</td>
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<tr>
<td>M. Avium</td>
<td>M. Avium</td>
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<tr>
<td>Candida</td>
<td>Candida</td>
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<tr>
<td>E. Biensuii (Microsporidia)</td>
<td>E. Biensuii (Microsporidia)</td>
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<tr>
<td>Cryptosporidium</td>
<td>Cryptosporidium</td>
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<tr>
<td>CMV</td>
<td>CMV</td>
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<tr>
<td>Pneumocystis</td>
<td>Pneumocystis</td>
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<tr>
<td>Toxoplasmosis</td>
<td>Toxoplasmosis</td>
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<tr>
<td>EBV ~ lymphomas</td>
<td>EBV - like lymphocryptovirus</td>
</tr>
<tr>
<td>PML - JC virus (polyomavirus)</td>
<td>PML - SV40 virus (polyomavirus)</td>
</tr>
</tbody>
</table>
Macaque models for transmission, pathogenesis, and prevention studies

**Asian macaque** (*Macaca mulatta*)
- a.k.a; *rhesus macaque*
- Indian or Chinese origin - similar transmissibility
- Seasonal breeders
- Advantage; availability, established protocols and reagents

**Pigtailed macaque** (*Macaca nemestrina*)
- Reportedly more susceptible to disease progression
- Year-round cycling
- Disadvantage; limited availability

**Cynomolgus macaque** (*Macaca fasicularis*)
- a.k.a. crab eating macaque, long tailed macaque
- Reportedly less susceptible to disease progression
- Advantage; available; smaller (less expensive)
- Disadvantage; microbicide applications not well established, small vaginal lumen volume - endoscopy limited

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, Daniel E. Shvetz, Douglas R. Pauley, Heather L. Knight, Michael Rosenzweig, R. Paul Johnson, Ronald C. Desrosiers, Andrew A. Lackner*
HIV Pathogenesis
HIV and SIV infection results in rapid and massive loss of intestinal CD4+ T cells

- Veazey, Lackner et al, Intestinal tract as a major site of CD4+ T cell depletion in early SIN infection; Science 1998.

CONFIRMED IN HUMANS 7 YEARS LATER:

The Mucosal Immune System:
Primary Target for HIV Infection and AIDS

Ronald S. Veazey and Andrew A. Lackner

Memory CCR5+ CD4+ T cells are selectively lost in all tissues in early infection.
Activation of CD4+ T cells drives viral replication

(Veazey et al, Trends in Immunol, 2001)

(a) **Before infection**
Inductive site
Effector site

- Ag exposure
- To mesenteric lymph node and blood
- Tissue expansion

Recirculation and homing

**Chronic infection, activation drives replication**

(b) **Peak viral replication (primary infect)**

- Ag exposure
- Decreased tissue expansion

- Decreased recirculation

**AIDS - tissue damage and OI driving viral replication**

(c) **Opportunistic infection established**

- Decreased tissue expansion

- Increased tissue expansion

- Increased Ag exposure and recirculation

Key:

- Naive (CCR5<sup>+</sup>) CD4 cells
- Naive CD8 cells
- Effector (CCR5<sup>+</sup>) CD4 cells
- Effector CD8 cells
- Resting, memory CD4 cells
- Ag-primed CD4 cells
- Ag-specific effector CD8 cells
- Ag-specific effector CD4 cells
- Antigen
- Active HIV replication
- T-cell receptor

TRENDS in Immunology
Importance of Activation and Differentiation of CD4+ T cells in AIDS Pathogenesis

Ronald S. Veazey and Andrew A. Lackner

Trends in Immunol 2002; 23:129

Mast cells: double agents of the immune system

T-cell regulation by Cbl and Cbl-c

Can TLRs sense different LPS shapes?

Prostaglandins, PPAR-γ and inflammation

CONFIRMED IN HUMANS 5 YEARS LATER: Brenchley, Douk et al.; Nat Med 2006: Microbial translocation is a cause of systemic immune activation in chronic HIV infection
Wang, Veazey et al., Simian immunodeficiency virus selectively infects proliferating CD4+ T cells in neonatal rhesus macaques, *Blood* 2010
Selected invited reviews and papers

Veazey RS and Lackner AA; HIV swiftly guts the immune system, Nature Medicine 11:469-70, 2005


Veazey RS; Mucosal immunopathogenesis of HIV infection: implications for vaccine development, Future HIV Therapy 1: 103-112, 2007

Veazey RS and Lackner, AA; The mucosal immune system and HIV-1 infection, AIDS Reviews, 5:245-52, 2003
Vaginal CD4⁺ T Cells Express High Levels of CCR5 and Are Rapidly Depleted in Simian Immunodeficiency Virus Infection

Ronald S. Veazey, Preston A. Marx, and Andrew A. Lackner
Tulane National Primate Research Center, Tulane University Health Sciences Center, Covington, Louisiana
Target cell (CD4+CCR5+ memory T cells) distribution in different tissue compartments (Gated through CD4+ Lymphocytes)

Optimal viral target cells reside in mucosal tissues (Veazey, Trends in Immunology, 2001)

Protection of macaques from vaginal SHIV challenge by an orally delivered CCR5 inhibitor

Nature Medicine, 11:1293-94, 2005
Phenotyping SIV infected cells in macaque intestines - Most infected cells in acute infection are CD4+CD45RO+ memory T cells

Phenotype of productively infected T cells in intestine

CD69 $+$ SIV (P28) $+$ CD3
Island Biogeography Reveals the Deep History of SIV

Michael Worobey,1 Paul Telfer,2,3 Sandrine Souquière,3 Meredith Hunter,2 Clint A. Coleman,2 Michael J. Metzger,2 Patricia Reed 2,3 Maria Makuwa,3 Gail Hearn,4 Shaya Honarvar,4 Pierre Roques,3 Cristian Apetrei,2 Mirdad Kazanjii,3 Preston A. Marx2*

20 AIDS Virus Has an Ancient History

HIV is a newcomer among human pathogens, having caused the first known cases of AIDS within the past few decades. So scientists suspected that SIV, the primate virus that spawned HIV, was just a few hundred years older. Tulane University virologist Preston Marx published research in September that suggests otherwise: SIV seems to be at least 32,000 years old, meaning it coexisted with people nearly all that time before HIV emerged.

Marx’s team did SIV tests on monkeys from Bioko Island, which was cut off from the African continent 10,000 years ago. The Bioko SIV strains all shared ancestry with strains from the African mainland, indicating the virus is at least that old and probably much older. “Events in the 20th century launched the virus from a benign monkey virus into a human epidemic,” Marx says. The growing use of blood transfusions and the rise of crowded cities may have helped pass SIV around and let it evolve into HIV.

If we do not figure out what triggered the HIV epidemic, it will be hard to prepare for what might come next. “We could be making new strains without knowing how to stop or control them,” Marx says. MONICA HEGREH
Non-progressing or “natural” primate hosts (African green monkeys and Sooty mangabeys) are naturally “missing” viral target cells in the intestine (Pandrea Veazey et al, Blood 2007)
Viral replication persists in intestinal inductive sites in SIV and SHIV infected macaques controlling viremia (<125 RNA copies/ml plasma).

Viral replication persists in GALT in HIV infected patients on ART

Intestinal CD4+ T cells are not fully restored in HIV patients on ART

Viral replication and continuing CD4+ T cell loss occurs in the gut, despite “HAART” or apparent “control” of plasma viremia.
Activated, memory CD4+CCR5+ T cells in mucosal tissues (intestines) are major targets for early SIV/HIV infection and amplification.

Activated (functional) CD4+CCR5+ memory cells predominantly reside in mucosal tissues.

Natural hosts have evolved? to become less reliant on (reduce or eliminate) CCR5+CD4+ T cells in tissues.

Mucosal cells play a major role in early HIV pathogenesis. Do CD4+ T cells play a role in transmission?
Progesterone implants enhance SIV vaginal transmission and early virus load. 

Mechanisms of vaginal HIV-1 transmission? Thinning or inflammation of the vaginal epithelium?

Vaginal biopsies from the same normal woman at peak follicular (A) and luteal (B) stage of menses. Note that the luteal phase has marked areas of thinning (open arrow) associated with dermal papillae (DP; arrows).

C shows marked inflammation and lymphocytic inflammation in the vaginal mucosa of a clinically “normal” woman.
ATTACHMENT OR FUSION INHIBITORS –
A SAFER APPROACH FOR MICROBICIDES?
Comparative histology of the vagina

Human vagina (luteal phase)

Macaque vagina (luteal phase)
Fusion inhibitors in clinical development; Mechnisms of action

Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120

RONALD S. VEAZEY, ROBIN J. SHATTOCK, MELISSA POPE, J. CHRISTIAN KIRJIAN, JENNIFER JONES, QINXUE HU, TOM KETAS, PRESTON A. MARX, PER JOHAN KLASSE, DENNIS R. BURTON & JOHN P. MOORE

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Immunotherapy

A microbicide for HIV?
Targeting T cells to tumors
Kaposi sarcoma and β-catenin
Blocking hyperglycemic damage
Alternative role for clotting factors in pregnancy
Prevention of Vaginal SHIV Transmission in Rhesus Macaques Through Inhibition of CCR5

Michael M. Lederman,† Ronald S. Veazey,‡ Robin Offord,§ Donald E. Mosier,⊥ Jason Dufour,∥ Megan Mefford,∥ Michael Piatak Jr.,∥ Jeffrey D. Lifson,∥ Janelle R. Salkowitz,† Benigno Rodriguez,† Andrew Blauvelt,§,‡ Oliver Hartley§

Topical agents, such as microbicides, that can protect against human immunodeficiency virus (HIV) transmission are urgently needed. Using a chimeric simian/human immunodeficiency virus (SHIV SF162), which is tropic for the chemokine receptor CCR5, we report that topical application of high doses of PSC-RANTES, an amino terminus–modified analog of the chemokine RANTES, provided potent protection against vaginal challenge in rhesus macaques. These experimental findings have potentially important implications for understanding vaginal transmission of HIV and the design of strategies for its prevention.
High dose of topical microbicides (mg/ml or mM) are required to prevent vaginal HIV-1 transmission

_PSC-RANTES dose titration results (Lederman, et al., Science 2004)_

Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion

Nature, 439: 99-102, 2005

BMS-378806: binds viral gp120 and prevents CD4 attachment;
CMPD167: binds to CCR5 to inhibit gp120 association;
C52L: peptide inhibitor of gp41-mediated fusion
Blocking either CD4 or CCR5 receptors can completely prevent vaginal HIV transmission

Implications for transmission, vaccine development?

CD4+CCR5+ cells are the first cells infected, and critically involved in establishment of HIV infection
Blocking CD4 or CCR5 binding alone can completely prevent vaginal transmission

Target cells for HIV transmission in the vagina, Poonia et al, 06

Dual-label immunohistochemistry for CD4 (red) and CCR5 (blue) in the vagina of normal (non-progestin treated) rhesus macaques. EPI=epithelium, LP=lamina propria
T cells in rhesus and human vagina

CD3 human vagina

CD3 rhesus vagina
Visualizing HIV in Tissue

Photoactivatable GFP labeled HIV

Before PA  |  After PA  |  p24  |  Merge

430nm
Virus Entry in Macaque Vagina

Ectocervix and vagina contain many virions

Virions enter interstitially

Virion penetration mimics human explant system
Studies of tight junctions and viral entry (Courtesy Tom Hope)

Macaque vagina GJ69 -Non-Depo
E-Cadherin
4B2 (Desmoglein 1&2)+ Zenon
DAPI
Various dendritic cell inhibitors have failed to protect macaques against SHIV challenge despite efficacy in *in vitro* systems.
Reservoir vs. Matrix Type Vaginal Rings

Cross-sectional profiles

Core-type

Matrix-type

TMC120 Raman maps

Map view

13 mm

Map view

15 mm

13 mm

15 mm

Courtesy of Karl Malcolm, QUB
Vaginal rings containing microbicides designed for macaques ongoing PK / PD studies of vaginal efficacy and safety

Malcolm, Veazey, Moore et al., Sustained release of the CCR5 inhibitors CMPD167 and maraviroc from vaginal rings in rhesus macaques, Antimicrobial Agents and Chemotherapy, 2012
Advances made through nonhuman primate research

The intestinal tract is the major site for initial HIV amplification and ongoing viral replication, even in “controllers” (discovered in macaques, confirmed in humans)

HIV-1 is a “machine” that requires “keys and fuel”; i.e., it infects and replicates in cells with appropriate receptors (keys), and that have a certain level of “activation” sufficient to promote mRNA translation; these viral “fuel” cells are “activated” CD4+CCR5+ memory cells mucosal tissues

Intestinal damage and systemic activation drive persistent HIV replication (discovered in macaques, confirmed in humans)

Non-progressing hosts have evolved to avoid AIDS by decreases dependence in CD4+CCR5+ cells for immunity; Nature could not cure this infection, so it changed the immune system of the host

Preservation (or restoration) of intestinal memory CD4+ T cells is the only correlate of protective immunity we have found to date

CD4+CCR5+ T cells in the vagina are the major source of vaginal transmission And infection can be completely prevented by blocking either receptor

• Nonhuman primate studies are essential and continue to guide all aspects of HIV research
Acknowledgements

Tulane National Primate Research Center
Xiaolei Wang
Huanbin Xu
Bapi Pahar
Terri Rasmussen
Jason Dufour
Preston Marx
Andrew Lackner

NIH / NIAID
Nancy Miller
Susan Plaeger
Roberta Black
Jim Turpin

Northwestern University, Chicago
Tom Hope

NIH / NIAID / NCRR
Jim Turppin
Karl Diffenbach
Opendra Sharma
Fulvia Veronese
Jack Harding

Case Western Reserve University
Michael Lederman
Eric Arts

Cornell University
John Moore
Pj Klasse

Harvard Medical School
Judy Lieberman
Keith Reimann
Norman Letvin
Joern Schmitz

St George’s Hospital, London
Robin Shatock

Queens University Belfast
Karl Malcolm

NCI-Frederick
Jeff Lifson
Mike Piatak