HIV Reservoirs in Developing Countries: Implication for HIV CURE Strategies

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Cissy Kityo¹; Thomas Schacker²; Francis Ssali¹; Jeffrey Chipman², Greg Bielman², Daniel Douek³; Peter Mugyenyi¹

1- Joint Clinical Research Centre
2- University of Minnesota
3- NIH Vaccine Research Centre
Regional differences in Absolute CD4 Counts
Population-Based Hematologic and Immunologic Reference Values for a Healthy Ugandan Population

Eric S. Lugada,1,2* Jonathan Mermin,2 Frank Kaharuza,2 Elling Ulvestad,3 Willy Were,2 Nina Langeland,4 Birgitta Asjo,3 Sam Malamba,2 and Robert Downing2

Center for International Health, University of Bergen,1 and Department of Microbiology and Immunology,3 and Department of Internal Medicine,4 Haukeland University Hospital, Bergen, Norway, and Centers for Disease Control and Prevention (CDC)—Uganda, Global AIDS Program, National Center for HIV, STD and TB, CDC, and Uganda Virus Research Institute, Entebbe, Uganda2


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<th>N</th>
<th>CD4 (cells/µl)</th>
<th>Range</th>
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T cell zone in lymphatic tissues

- Homeostasis of T cells
- Cell movement
- Cytokine diffusion
Inflammation is important because:

- Studies by our group have shown that virus replication in the LN causes an inflammatory reaction that produces a form of fibrosis in the lymph node. This damages mechanisms that maintain the CD4 T count.
HIV replication
  ↓
Inflammation
  ↓
Fibrosis
  ↓
Impairs
    ↓
cell movement
    ↓
cytokine diffusion
    ↓
access to nutrients
  ↓
T Cell depletion
Quantitative Image Analysis of Collagen Tissues in LT

Schacker, et al, JCI, 2002
TZ CD4 T Cell Population

HIV negative

HIV positive
Lymphoid Populations of CD4+ T cells

LN

PP

LP

HIV -

HIV +
The size of the CD4 population in LN correlates to the amount of collagen in the TZ

\[ R^2 = 0.72, \ P < 0.0001 \]

Schacker, et al, JCI, 2002
Amount of lymphatic tissue fibrosis in HIV infection predicts magnitude of HAART-associated change in peripheral CD4 cell count

Timothy W. Schacker\textsuperscript{a}, Cavan Reilly\textsuperscript{b}, Gregory J. Beilman\textsuperscript{c}, Jodie Taylor\textsuperscript{c}, David Skarda\textsuperscript{c}, David Krason\textsuperscript{a}, Matthew Larson\textsuperscript{a} and Ashley T. Haase\textsuperscript{d}

AIDS, 2005, 19 (18); 2169-2171
Viral Replication

Other Infections

Microbial Translocation

Inflammation

Collagen Deposition (Cumulative)

Impedes Access

FRC Network Source

IL-7

Decreases

LTβ+ T Cells

Increases

T Cell Apoptosis

Depletes

T Cell Populations

Depletes
Study of HIV associated lymph node fibrosis and viral reservoirs in Uganda
Rationale

Viral reservoirs and factors that maintain these reservoirs are currently under study in the United States and Europe, but very little is known about viral dynamics in resource limited settings where the infection is taking its greatest toll.
Hypothesis

• The size of HIV RNA and DNA reservoirs will be greater in East Africa because of increased inflammation and immune activation

  Lifelong exposure to pathogens like TB, malaria, helminths etc leads to higher levels of T cell activation
Objectives

• To measure the amount of LT fibrosis and frequency of HIV RNA and HIV DNA positive cells in HIV+ individuals in Kampala Uganda both before and during antiretroviral therapy.

• Compare measures of lymphatic tissue architecture from tissues obtained in Kampala to age matched controls in Minnesota (HIV+ tissue specimens will be matched by stage of disease as well).

• To measure the size of the vRNA and vDNA reservoir in patients from Uganda for comparison to similar subjects from the west.
Joint Clinical Research Center and University of Minnesota Collaboration

- 105 HIV+ and HIV- people studied
- 228 LN biopsies
- 145 rectal biopsies
Study design

• Cross sectional cohort of HIV+ and HIV- participants in Uganda and the US
• Prospective cohort of HIV+ participants initiated on ART
## Protocol

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<td>Plasma Viral Load (copies/ml)</td>
<td>CD4 T cell count (cells/µl peripheral blood)</td>
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Average: 32

Average Plasma Viral Load: 81,827 copies/ml

Average CD4 T cell count: 174 cells/µl
Tissue Analyses:

- Immune activation (Ki67)
- Amount of LN fibrosis before and during therapy
- Size of the population of LN CD4 T cells
- Frequency of vRNA+ cells before and during therapy
- Frequency of vDNA+ cells before and during therapy
Identification of vRNA and vDNA+ cells by in situ hybridization
Ki67 Staining as a Marker for Immune Activation

Minnesota HIV-

Uganda HIV-
Ki67 Staining as a Marker for Immune Activation

Minnesota HIV+

Uganda HIV+
HIV negative Ugandans have significantly increased levels of immune activation compared to HIV negative individuals in Minnesota, USA.
Trichrome stain of lymph node sections to identify collagen fibers

United States

HIV -

HIV +

Uganda
The area of the LN occupied by collagen does not decrease with long-term ART.
Reciprocal relationship between collagen in LN and the population of CD4 T cells in HIV+ and HIV- populations in the U.S. and Uganda.
TZ Collagen and CD4+ T Cell Populations in Lymph Nodes From Uganda
In Uganda

• Measures of immune activation are increased in the HIV negative population

• There is increased lymphatic fibrosis in the HIV negative population

• There is CD4 T cell depletion in LN of HIV negative Ugandans

• HIV associated lymphatic fibrosis does not decrease with ART

What about reservoir size?
Frequency of vRNA+ cells/gram LT before ART
Frequency of vDNA+ cells/gram LT before ART
The ratio of vRNA+ to vDNA+ cells is ~.002
How many vDNA+ cells are able to produce virus?

Intact virus genome

Viral outgrowth assay

DNA+

Ho, et al, Cell, 2013
“Back of the envelope” calculations for the size of the vDNA+ cellular reservoir

\[
\frac{10^9 \text{ cells}}{\text{gram}} \times \frac{1000 \text{ g}}{\text{Kg}} \times \frac{70 \text{ Kg}}{\text{human}} \times 0.01 \text{ kg LT}
\]

10% inducible: \(7 \times 10^{11}\) inducible vDNA+ cells/human

5% inducible: \(3.5 \times 10^{11}\) inducible vDNA+ cells/human
There is an initial 2 log decay in vDNA+ cell with ART and then it remains stable over time (i.e., ~ 7 x 10^9 inducible cells)
Conclusions

- HIV negative persons have increased immune activation and LN pathology that resembles early HIV infection in the U.S.
- T cell populations are relatively depleted at the time the individual becomes HIV infected
- HIV infected LN in Uganda have similar fibrotic changes to people with chronic infection in the U.S. that do not resolve with ART
Conclusions

• The size of the vRNA+ cell reservoir is quite large in untreated infection and resolves promptly with ART (as it does in the U.S.)

• The size of the vDNA+ cell population is unexpectedly large, especially the inducible population of cells

• There is an initial 2 log decay in vDNA+ cells and then the reservoir remains stable at $\sim 10^9$ cells.
Approaches to HIV Cure

Drugs that reactivate HIV-infected resting cells

*Latency reversing agents*

Genetic modification of CD4+ T cells to prevent HIV entry and replication

*Zinc-finger nucleases: delete part of CCR5 co-receptor*

Stem cell transplantation

*donor stem cells lacking CCR5 to replace the immune system*

Boosting the immune system to kill residual virus expressing cells

*Therapeutic vaccines; Broadly neutralizing antibodies*

Early ART initiation to limit the size of the reservoir
Does LT Fibrosis and Reservoir size matter?

- A larger reservoir means greater & more persistent immune activation leading to LT fibrosis and reduced immune reconstitution
  - This may account for differences in responsiveness to immune therapy strategies for different populations

- Fibrosis may limit diffusion of therapeutic agents into the LN where the virus replicates

- Patients with large reservoirs may need additional combinations than those with smaller reservoir sizes

- Therefore HIV eradication and cure strategies contemplated for western populations may not apply to populations in the developing world
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