HIV Antibodies as Markers of HIV Systemic Reservoir and Viral Suppression

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No current blood assay accurately quantifies the systemic reservoir.
Can Host Immune Responses Against HIV Be Used to Quantify The Latent HIV Reservoir?

HIV Ab levels, avidity, specificities...

Peripheral Reservoir Measures

Tissue Reservoir Measures

Intrinsic antiviral restriction factors
Using Seroreversion as a Marker of Viral Suppression

The Effects of Early Antiretroviral Therapy and Its Discontinuation on the HIV-Specific Antibody Response

M. SCOTT KILLIAN, PHILIP J. NORRIS, BHUPAT D. RAWAL, MILA LEBEDEVA, FREDERICK M. HECHT, JAY A. LEVY, and MICHAEL P. BUSCH

FIG. 1. Representative cases. Shown are antibody levels (closed circles), virus titers (open squares), and CD4+ T cell counts (open triangles) for three representative subjects. Data are provided for (A) a therapy naive subject who exhibited an indeterminate Western blot result at week 0 and chose not to receive ART; (B) a subject undergoing primary HIV-1 infection and initiated ART upon entry into the study; and (C) a subject who exhibited negative Western blot and standard EIA test results at week 0, initiated ART after 35 weeks of observation, and then 60 weeks later, discontinued therapy.
Less Sensitive HIV-1 Enzyme Immunoassay as an Adjuvant Method for Monitoring Patients Receiving Antiretroviral Therapy

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FIG. 1. Mean optical densities (ODs; quantified to the left) and viral loads (log_{10}, quantified to the right) in the group of 20 patients treated with antiretroviral drugs (Spearman = 0.224; p = 0.009)
Evidence for Persistent Low-Level Viremia in Individuals Who Control Human Immunodeficiency Virus in the Absence of Antiretroviral Therapy

Hiroyu Hatano,1,* Eric L. Delwart,1,2 Philip J. Norris,1,2 Tzong-Hae Lee,2 Joan Dunn-Williams,2 Peter W. Hunt,1 Rebecca Hoh,1 Susan L. Stramer,3 Jeffrey M. Linnen,4 Joseph M. McCune,1 Jeffrey N. Martin,1 Michael P. Busch,1,5 and Steven G. Deeks1

FIG. 1. In vitro spiking experiments showing the relationship between HIV RNA levels and S/Co ratio using the TMA assay. Each dot represents the mean of the results for four replicates (randomly selected from 20 replicates) performed by four different laboratory technicians; the lines represent the mean S/Co ratio for each viral load copy number (0.1 5 10 30 100 and 300 copies/ml).

FIG. 3. Baseline plasma HIV RNA level for 37 HAART-suppressed subjects and 46 elite controllers (EC). The lines represent the median S/Co ratio for each group.
Limited Seroconversion Following Early ART Based on Vitros LS and Avidity Assays

FIG 3 Seroconversion panels from treated and untreated subjects who were monitored for >300 days after infection. Seroconversion panels from 9 untreated and 9 treated individuals were monitored for evolution of LS and avidity-modified Vitros results over the first 500 days of infection. (A) All 9 untreated individuals reach the selected cutoff values for LS (S/C ratio of 20) and avidity (AI of 0.6) Vitros assays. (B) However, the threshold values for Vitros assays are not reached for 7 of the 9 individuals.
Reduction in HIV Ab reactivity in EC and Following ART in CEPHIA

Keating et al. CROI 2014
The Berlin Patient: HIV Eradication

Yukl et al. Plos Pathogens 2013
Changes in HIV Ab levels following allogeneic (CCR5-expressing) HSC transplant in 2 HIV+ pts who had absence of HIV RNA and DNA detection post-transplant (on HAART during and after HSC transplant)

LAg Assay

LS-VITROS Assay

Timothy Henrich & Daniel Kuritzkes, IAS 2012&IAS 2013
Journal of Infectious Diseases 2013
DARE Supplement: “Application of HIV antibody assays to quantify humoral immune responses as measures of viral persistence and systemic HIV reservoir”

- UCSF “Early vs Late Treatment (ELT)”: ART treated during acute or during chronic infection.
- Treat Elite: ECs with HAART demonstrating significant reduction in plasma and gut biopsy VL by ultrasensitive methods as well as reduction in immune activation.
- Participants with long-term ART suppression with and without intermittent viremia (“Ab set-points”).
- Cure protocols: Longitudinal series of samples from multiple patients enrolled in:
  - Hematopoietic stem cell transplants
  - HAART intensification protocols
  - “Shock and kill” (Vorinostat, Disulfiram, Panobinostat)
  - Other Cure strategies
Early and Late ART (ELT) Cohort

60 Options participants w/ 5 serial plasma samples
30 were treated during acute and 30 during chronic infection
HIV Abs in ELT Cohort

LS-VITROS - Early Treated

AVIDITY VITROS - Early Treated

LAG - Early Treated

LS-VITROS - Late Treated

AVIDITY VITROS - Late Treated

LAG - Late Treated
Pre-treatment HIV-Ab Correlates with VL

**LAg**

- Log ODn vs. Log Viral Load (copies/mL)
- $p < 0.001$
- $r^2 = 0.32$

**LS-VITROS**

- Log S/Co vs. Log Viral Load (copies/mL)
- $p < 0.001$
- $r^2 = 0.23$

**Avidity VITROS**

- Log Avidity Index vs. Log Viral Load (copies/mL)
- $p = 0.07$
- $r^2 = 0.01$
Stable HIV Ab reactivity in Viremic and Elite Controllers
Post-Treatment Ab Correlates with Viral Reservoir

Limiting Antigen Assay

Log Ab vs Log Proval DNA

(p=0.01, r^2=0.1)

Limiting Antigen Assay

Log Ab vs Log Cell-Associated RNA

(p=0.04, r^2=0.06)

LS-VITROS

Log Ab vs Log Proval DNA

(p=0.01, r^2=0.1)

LS-VITROS

Log Ab vs Log Cell-Associated RNA

(p=0.02, r^2=0.09)

VITROS Avidity

Log Ab vs Log Proval DNA

(p=0.05, r^2=0.06)

VITROS Avidity

Log Ab vs Log Cell-Associated RNA
Host restriction factors attack HIV within the infected cell

Many steps of the HIV-1 life cycle are targeted by intrinsic antiviral factors. HIV-1 has evolved strategies to counteract these intrinsic antiviral factors, through accessory proteins or other unknown mechanisms that are currently under investigation.

Santa-Marta et al, 2013
Host restriction factor expression and HIV persistence

LAG Ab Correlates with Host Restriction Factors during ART

Figure 5

AIDS 2015, 29:411–420

<table>
<thead>
<tr>
<th>HIV antibodies (All)</th>
<th>HIV antibodies (Chronic Tx)</th>
<th>HIV antibodies (Early Tx)</th>
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<tr>
<th>Cell-associated HIV RNA</th>
<th>Cell-associated HIV DNA (pol)</th>
<th>Transcriptional ratio (HIV RNA / HIV DNA (pol))</th>
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<thead>
<tr>
<th>CD3+CD4+HLA-DR+CD38+ percentage</th>
<th>CD3+CD4+PD-1+ percentage</th>
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<td>Positive correlation p&lt;0.001</td>
<td>Negative correlation p&lt;0.001</td>
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Diagram:
- Entry and uncoating
- Reverse transcription
- Integration
- Translation
- Assembly
- Budding
LS-VITROS

Days from Initial Blood Draw

S/C O

Avidity VITROS

Avidity Index

Days from Initial Blood Draw

ACTG 5321
Antibodies after disulfram
Reservoir Assay Validation and Evaluation Network (RAVEN)

Create large repository of pedigreed specimens from ART-suppressed participants to be used to identify/qualify biomarkers to detect HIV persistence

Identify high-throughput, blood-based assays that can be validated and advanced as biomarkers in clinical trials to characterize HIV reservoir & predict the time to rebound post ATI.

UCSF/BCP

Prospective apheresis collection from Clade B HIV+ donors

HIV+ Participants in Options/SCOPE on ART

BSRI/SANBS

Processing
Characterization
Panel building and distribution

Testing labs

SANBS

Prospective apheresis collection from Clade C HIV+ donors

QVOA evaluation module

HIV+ NAT-yield & recent SC donors on ART

Analysis of tissue reservoirs
Immunologic approaches: HIV Abs, Intrinsic immune/restriction factors

Plasma HIV RNA Quantification
PBMC HIV DNA/RNA Quantification
CD4+ Memory T cell HIV DNA/RNA

Sequence-based approaches
Flow cytometry
PACS
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CEPHIA