HIV/STDs and Excretion of HIV in Genital Secretions
- Implications for sexual transmission

Pietro L. Vernazza,
KSSG, St. Gallen, Switzerland
Where it all began...
ISOLATION OF HTLV-III/LAV FROM CERVICAL SECRETIONS OF WOMEN AT RISK FOR AIDS

MARKUS W. VOGT    DAVID J. WITT
DONALD E. CRAVEN    ROY BYINGTON
DAVID F. CRAWFORD    ROBERT T. SCHOOLEY
MARTIN S. HIRSCH

Infectious Disease Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114; Department of Medicine, Division of Infectious Diseases, Boston City Hospital; Boston University School of Medicine, Boston, MA 02118, USA
New study: AIDS virus carried by women

By MICHAEL LASALANDRA

The deadly AIDS virus for the first time has been found in the genital secretions of women, increasing evidence that it can be spread sexually from women to men, researchers said yesterday.

While the study did not prove that women can sexually transmit the virus, it warned that contact with prostitutes or anonymous sexual activity — either

Key find in treatment of disease

WASHINGTON — In a development that may lead toward a treatment for AIDS, researchers reported yesterday they have isolated an enzyme that controls the ability of the AIDS virus to infect people.

The availability of this enzyme in the laboratory means that scientists will be able to test a variety of drugs against it to find the best way to block its activity and thus keep the AIDS virus from infecting normal cells.

Several drugs are being studied for possible activity against the deadly Acquired Immune Deficiency Syndrome but there is as yet no way to stop the progression of the disease.
Schultz et al. BMJ 1999;319:1596-1600
VL and HIV-Transmission-Risk

- Rakai (Uganda)
- 453 HIV-disc. couples
- 11.6 % TR / year

HIV-RNA in blood and semen

Vernazza, 2000, Quest-Study Group
Genital and blood VL in women

\[ r = 0.52 \]
\[ p = 0.0001 \]

Viral load in blood vs. semen during the course of treatment in PHI

- **RNA blood**
- **RNA seminal fluid**
- **DNA seminal cells**

<table>
<thead>
<tr>
<th>Time</th>
<th>N</th>
<th>HIV-RNA (log₁₀ cp/ml)</th>
<th>HIV-DNA (log₁₀ c/Ejac)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>32</td>
<td>6.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Early</td>
<td>22</td>
<td>5.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Late</td>
<td>22</td>
<td>4.0</td>
<td>2.5</td>
</tr>
<tr>
<td>CAD</td>
<td>39</td>
<td>3.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Acute & Primary HIV Infection**

**CAD**
Summary 1

- HIV in genital tract follows blood VL

→ Q: Is there a genital compartment
→ Q: What is the effect of treatment
HIV in semen under HIV therapy

>1 log drop

<0.5 log drop

Vaginal HIV under HIV therapy

Hart C. et al, JID, 1999;179:871-82
HIV in Semen during HAART

Vernazza et al, AIDS, 2000, 14;117-21
HAART: Latent HIV in seminal cells

- 28 men
- Suppressive HAART
- PBMC and SC
  - Viral growth
  - 2-LTR-circular DNA

QUEST:

HIV-RNA in semen during tx

![Graph showing HIV-RNA levels in semen and plasma over days on therapy. The graph includes data points for Plasma RNA and Semen RNA, with a notable outlier at #6991.](image)
QUEST:

HIV-DNA in semen during tx

HIV-DNA (log_{10} / Ejac.)

Viral DNA / ejaculate

Plasma RNA

Days on Therapy

#6991
HIV RNA Detected in a Genital HSV Lesion During Rx with Acyclovir

Courtesy slide: L. Corey
Malawi urethritis project: HIV-RNA in semen

Local inflammation and genital shedding of HIV: Men

Table 3. Significant ($P \leq 0.05$) biological correlates of HIV shedding in the male genital tract in studies published between 1996 and 2002.

<table>
<thead>
<tr>
<th>Correlate</th>
<th>Endpoint</th>
<th>Study population</th>
<th>Study type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic urethritis</td>
<td>RNA</td>
<td>UK</td>
<td>Cross-sectional</td>
<td>[154]</td>
</tr>
<tr>
<td>Symptomatic urethritis</td>
<td>RNA</td>
<td>Malawi</td>
<td>Longitudinal</td>
<td>[148]; (reviewed in [120])</td>
</tr>
<tr>
<td>Genital ulcer disease</td>
<td>Genetic diversity</td>
<td>Malawi</td>
<td>Longitudinal</td>
<td>[152,153]</td>
</tr>
<tr>
<td><em>Trichomonas vaginalis</em></td>
<td>RNA</td>
<td>Malawi</td>
<td>Cross-sectional</td>
<td>[149]</td>
</tr>
<tr>
<td>Seminal polymorphonuclear cell</td>
<td>Culturable virus</td>
<td>US</td>
<td>Observational</td>
<td>[147]</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>RNA Culturable virus</td>
<td>US</td>
<td>Observational</td>
<td>[147]</td>
</tr>
</tbody>
</table>
Tx of Cervicitis and Shedding of HIV

McClelland et al, AIDS 2001; 15:105-110
Tx of Cervicitis and Shedding of HIV

McClelland et al, AIDS 2001; 15:105-110
Local inflammation and genital shedding of HIV: Women

Table 2. Significant ($p \leq 0.05$) microbiological correlates of HIV-1 shedding in the female genital tract in studies published between 1996 and 2002.

<table>
<thead>
<tr>
<th>Correlate</th>
<th>Endpoint</th>
<th>Study population</th>
<th>Study type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial vaginosis</td>
<td>RNA</td>
<td>US</td>
<td>Cross-sectional</td>
<td>[287]</td>
</tr>
<tr>
<td>Herpes simplex virus-2</td>
<td>RNA</td>
<td>Central Africa</td>
<td>Cross-sectional</td>
<td>[118]</td>
</tr>
<tr>
<td>Human papillomavirus</td>
<td>Cell-free and cell-associated RNA, not DNA</td>
<td>Italy</td>
<td>Cross-sectional</td>
<td>[176]</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td>Detectable RNA</td>
<td>Côte d'Ivoire</td>
<td>Cross-sectional</td>
<td>[128]</td>
</tr>
<tr>
<td></td>
<td>Swab DNA and RNA</td>
<td>Kenya</td>
<td>Treatment</td>
<td>[129]</td>
</tr>
<tr>
<td></td>
<td>Swab RNA and DNA</td>
<td>Kenya</td>
<td>Treatment</td>
<td>[130]</td>
</tr>
<tr>
<td></td>
<td>Multiple</td>
<td>Africa</td>
<td>Meta-analysis</td>
<td>[119]</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>Detectable RNA</td>
<td>Côte d'Ivoire</td>
<td>Cross-sectional</td>
<td>[128]</td>
</tr>
<tr>
<td></td>
<td>DNA swab</td>
<td>Kenya</td>
<td>Cross-sectional</td>
<td>[127]</td>
</tr>
<tr>
<td></td>
<td>Swab DNA and RNA</td>
<td>Kenya</td>
<td>Treatment</td>
<td>[129]</td>
</tr>
<tr>
<td></td>
<td>Multiple</td>
<td>Multiple</td>
<td>Meta-analysis</td>
<td>[120]</td>
</tr>
<tr>
<td><em>Candida vulvovaginitis</em></td>
<td>DNA</td>
<td>Kenya</td>
<td>Cross-sectional</td>
<td>[127]</td>
</tr>
<tr>
<td></td>
<td>Swab RNA and DNA</td>
<td>Kenya</td>
<td>Treatment</td>
<td>[130]</td>
</tr>
<tr>
<td>Non-specified ulcers and/or inflammation</td>
<td>Cell-free and cell-associated CVL RNA</td>
<td>US</td>
<td>Treatment</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>CVL RNA</td>
<td>US</td>
<td>Treatment</td>
<td>[137]</td>
</tr>
<tr>
<td></td>
<td>Vaginal biopsy DNA and RNA</td>
<td>Thailand</td>
<td>Cross-sectional</td>
<td>[160]</td>
</tr>
<tr>
<td></td>
<td>DNA</td>
<td>Kenya</td>
<td></td>
<td>[126]</td>
</tr>
</tbody>
</table>

*Many of the analyses assess the contribution of multiple or any co-factor to genital HIV shedding. CVL, cervicovaginal lavage.
Effect of HSV on genital HIV

- 200 women, HSV-2 and HIV seropos.
  - 71 with detectable cervical HSV-2
- ↑ cervical HSV-level (per log10) assoc. w.
  - ↑ HIV-RNA 1.35-fold (1.0-1.81)
  - ↑ HIV-DNA 1.36-fold (1.05-1.75)
Urethritis during HAART (n=24)

Plasma HIV-RNA

Semen HIV-RNA

2 / 20 positive (low level)

4 / 4 positive (high level)

Summary 2

- HIV in genital tract follows blood VL
- Compartmentalized HIV-shedding possible with local inflammation

Q: Contribution of cells vs. free HIV in STDs?
PHI Transmission couple

22 y/o woman

AZT+3TC+ABC+APV

HIV-Ag  -  -  ++

C.trach.-PCR  -  +  +

24 y/o man

Blood plasma

Seminal plasma

Seminal cells (DNA)
HIV-DNA and RNA in semen during PHI (QUEST-Study)
Viral load in female genital tract

Andreoletti, JID, 2003;189:549-54
Viral load in female genital tract

$\text{Genital RNA}$

$\text{Blood RNA}$

$r = .50$

Andreoletti, JID, 2003;189:549-54
Viral load in female genital tract

Andreoletti, JID, 2003;189:549-54
Viral load in female genital tract

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Correlation between genital RNA and DNA.}
\end{figure}

Andreotti, JID, 2003;189:549-54
To explore the mechanism of sexual transmission of human immunodeficiency virus type 1 (HIV-1), we compared HIV-1 gp120 sequences in longitudinal samples from five acute seroconvertors with those from their corresponding sexual partners (transmitters). We used a quantitative homoduplex tracking assay to compare the overall genetic composition of HIV-1 quasispecies in each transmission pair and to track the transmitted viruses during the acute and asymptomatic stages of HIV-1 infection. In the chronically infected transmitters, HIV-1 variants in genital secretions differed from those in blood and variants in cells differed from those in cell-free plasma, indicating remarkable sequence heterogeneity in these subjects as well as compartmentalization of the virus in different bodily sites. Conversely, two of five seroconvertors had only a few related variants and three of five harbored only one viral population, indicating that in these subjects the transmitted viruses were typically homogeneous. Transmitted viruses were evident in the donor’s seminal plasma (one of five cases) and even more so in their seminal cells (three of five cases), suggesting that both cell-associated and cell-free viruses can be transmitted. In every pair studied, the transmitted variant(s) represents only a minor population in the semen of the corresponding transmitter, thereby providing evidence that HIV-1 selection indeed occurs during sexual transmission.
Cell-free vs. Cell-associated trm

Gender differences in HIV-1 diversity at time of infection

E. Michelle Long1,2,3, Harold L. Martin, Jr.4, Joan K. Kreiss4,
Stephanie M.J. Rainwater1,3, Ludo Lavreys4, Denis J. Jackson4,5, Joel Rakwar5,
Kishorchandra Mandalia6 & Julie Overbaugh1,3

1Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA
2Molecular and Cellular Biology Program, 3Departments of Microbiology,
4Medicine and Epidemiology, University of Washington, Seattle, Washington, USA
5Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya
6Coast Provincial General Hospital, Mombasa, Kenya.
Correspondence should be addressed to J.O.; email: joverbun@fhcrc.org

HIV diversity develops over time

6 mt

infection

time
HIV diversity in early infection

• Men: 1 pattern
  → <5% diversity: homogenous pattern

• Women: 2 patterns
  → homogenous pattern
  → heterogenous pattern
Potential hypotheses

• Infected by different partners
  – Similar # sex acts / wk
  – Similar fraction with single partner
  – Phylogenetic clustering
• Differences in immune response
  – 5/6 w already heterogenous before s/c
• Difference in transmitted virus
Hypothesis

- Sex. Trsm of HIV = rare event $\rightarrow$ 1 hit
- One single hit could be
  - Free virus
  - HIV-infected cell
- Multiple strains $\rightarrow$ Cell-associated
## HIV-diversity, STDs and DNA

### Table 2. Association between HIV-1 genetic complexity and factors present at the time of infection.

<table>
<thead>
<tr>
<th></th>
<th>Heterogeneous proportion (%)</th>
<th>Homogeneous proportion (%)</th>
<th>OR (95% CI)$^a$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial vaginosis</td>
<td>41/88 (47)</td>
<td>27/65 (41)</td>
<td>1.2 (0.6–2.6)</td>
<td>0.5</td>
</tr>
<tr>
<td>Candida vaginitis</td>
<td>27/88 (31)</td>
<td>13/67 (19)</td>
<td>1.8 (0.8–4.2)</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Chlamydia</em></td>
<td>2/74 (3)</td>
<td>1/57 (2)</td>
<td>1.6 (0.1–93.4)</td>
<td>1.0</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>6/89 (7)</td>
<td>9/67 (13)</td>
<td>0.5 (0.1–1.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>Genital ulcer disease</td>
<td>4/89 (4)</td>
<td>4/67 (6)</td>
<td>0.7 (0.1–4.2)</td>
<td>0.7</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>12/88 (14)</td>
<td>10/67 (15)</td>
<td>0.9 (0.3–2.4)</td>
<td>0.8</td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td>30/89 (34)</td>
<td>13/67 (19)</td>
<td>2.1 (0.9–4.8)</td>
<td>0.05</td>
</tr>
<tr>
<td>Cervicitis</td>
<td>23/88 (30)</td>
<td>7/66 (11)</td>
<td>3.0 (1.1–8.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cervical mucopus</td>
<td>9/89 (10)</td>
<td>3/67 (4)</td>
<td>2.4 (0.6–14.3)</td>
<td>0.2</td>
</tr>
<tr>
<td>Vulvitis</td>
<td>7/74 (9)</td>
<td>3/53 (6)</td>
<td>1.7 (0.4–10.9)</td>
<td>0.5</td>
</tr>
<tr>
<td>Any genital infection$^d$</td>
<td>52/57 (91)</td>
<td>29/42 (69)</td>
<td>4.7 (1.4–18.1)</td>
<td>0.005</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>21/51 (41)</td>
<td>11/49 (22)</td>
<td>2.4 (0.9–6.4)</td>
<td>0.04</td>
</tr>
<tr>
<td>Depo-medroxyprogesterone$^e$</td>
<td>35/65 (54)</td>
<td>15/53 (28)</td>
<td>3.0 (1.3–6.9)</td>
<td>0.005</td>
</tr>
<tr>
<td>Any hormonal contraception$^e$</td>
<td>56/86 (65)</td>
<td>26/64 (40)</td>
<td>2.7 (1.3–5.6)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*Sagar, AIDS, 2004;18:615-9*
Summary 3

- HIV in genital tract follows blood VL
- Compartementalised HIV-shedding possible with local inflammation
- Cell-associated HIV-DNA needs more attention
- STD-associated transmission might be more cell-associated