Cryptic transcription of HIV-RNA species from “defective” proviruses: A novel pathway for persistent immune activation in patients with HIV-1 infection and mechanism for persistent seropositivity despite “undetectable” levels of virus

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NIAID, NIH, DHHS; Leidos Biomedical Research, Inc.; Pacific Biosciences
Conflict of interest

The authors declare no competing financial interests.
Despite years of successful therapy (defined as HIV-RNA levels <50 copies/ml), the majority of HIV-infected patients exhibit persistent seropositivity to HIV-1 and evidence of chronic immune activation.

While plasma HIV-RNA levels are often referred to as “undetectable”, cell-associated proviral DNA can still be detected in circulating PBMCs. Much of this proviral DNA has been characterized as “defective”.

We have recently reported that such “defective” proviral DNA is capable of transcribing RNA. (Imamichi et al., AIDS, 2014;28:1091)
Interestingly, in the setting of bone marrow transplantation, a loss of seropositivity has been reported in association with a loss of proviral DNA.

- Hütter et al., NEJM, 2009;360:692
- Henrich et al., JID, 2013;207:1694
- A patient treated at the NIH Clinical Center (unpublished data)
Purpose

Determine whether or not the peripheral blood pool of proviral DNA is capable of being transcribed and translated into viral proteins even if unable to encode an intact virus.
Study design

1. Examination of proviral DNA and cell-associated RNA from the same cell source

   - Cryopreserved PBMCs from HIV+ patients
   - CD4+ T cells (negatively selected)
   - Co-purification of DNA/RNA
   - Single genome amplicons (SGAs) by end-point limiting dilution PCR (U5 to U5)
   - Sequencing

2. Examination of proviral DNA from cells expressing HIV-1 proteins

   - H9/HTLV-III_MN cells
   - Flow sorted into:
     1. Gag+gp120+
     2. Gag+gp120-
     3. Gag-gp120-
   - Purification of DNA
   - Single genome amplicons (SGAs) by end-point limiting dilution PCR (U5 to U5)
   - Sequencing
## Study participants

<table>
<thead>
<tr>
<th></th>
<th>Yrs. since 1st tested</th>
<th>ART regimen</th>
<th>HIV-RNA (copies/ml)</th>
<th>CD4⁺ (cells/µl)</th>
<th>Yrs. of HIV-RNA &lt;50</th>
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<tr>
<td>Pt 1</td>
<td>12</td>
<td>3TC+d4T+RTV</td>
<td>&lt;50</td>
<td>796</td>
<td>4</td>
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<td>Pt 2</td>
<td>16</td>
<td>3TC+d4T+RTV</td>
<td>&lt;50</td>
<td>879</td>
<td>4</td>
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<tr>
<td>Pt 3</td>
<td>19</td>
<td>FTC+TDF+EFV</td>
<td>&lt;40</td>
<td>1,061</td>
<td>6</td>
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<tr>
<td>Pt 4</td>
<td>15</td>
<td>3TC+AZT+NFV</td>
<td>&lt;50</td>
<td>675</td>
<td>4</td>
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<tr>
<td>Pt 5</td>
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<td>FTC+TDF+RTV+ATV</td>
<td>&lt;40</td>
<td>1,142</td>
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<td>Pt 6</td>
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<td>FTC+TDF+NVP</td>
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<td>Pt 8</td>
<td>n.a.</td>
<td>-</td>
<td>&gt;500,000</td>
<td>82</td>
<td>-</td>
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</table>
High prevalence of truncated “defective” proviruses in a patient with prolonged viral suppression

Patient 1
HIV-RNA <50
CD4+ = 796

Near full-length

Truncated

X In-frame stop codon
Clonal expansion of truncated “defective” proviruses

Patient 2

HIV-RNA <50
CD4+ = 879

Clonal expansion

3.3 kb 1
3.3 kb 1
3.3 kb 1
3.3 kb 1
3.3 kb 1
3.3 kb 1
3.3 kb 1
3.3 kb 1
3.3 kb 1
3.3 kb 1
2.2 kb 1
2.0 kb 1
8.9 kb 9
2.7 kb 1
Frequencies of proviruses with different lengths

<table>
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<th>Length</th>
<th>Freq.</th>
<th>Number of open reading frames</th>
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<tr>
<td>&gt; 8 kb</td>
<td>6%</td>
<td>8-9</td>
</tr>
<tr>
<td>6-8 kb</td>
<td>11%</td>
<td>5-7</td>
</tr>
<tr>
<td>4-6 kb</td>
<td>29%</td>
<td>1-3</td>
</tr>
<tr>
<td>2-4 kb</td>
<td>42%</td>
<td>1</td>
</tr>
<tr>
<td>&lt; 1 kb</td>
<td>12%</td>
<td>0</td>
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</tbody>
</table>

n=237 (7 pts)
The truncated “defective” proviruses are associated with novel RNA transcripts.

Patient 8
- HIV-RNA >500,000
- CD4+ = 82

Patient 3
- HIV-RNA <40
- CD4+ = 1,061
H9 cells infected with HIV\textsubscript{MN} are capable of expressing HIV-1 proteins in the absence of intact proviruses.
Summary

Utilizing an LTR to LTR “full-length” PCR, we identified a high prevalence of “defective” proviruses in patients with prolonged viral suppression.

These proviruses:

- Contain large internal deletions
- Are associated with RNA transcription
- Are capable of encoding viral proteins in the absence of an intact virus
- Could be found in cells undergoing clonal expansions and thus were associated with long-term persistence
These “defective” proviruses:
- Are incapable of producing intact viruses
- Can still inflict harm by producing foreign proteins or by recombining to form intact viruses

These results may help to explain:
- The persistent seropositivity in most patients with controlled HIV-1 infection
- The sero-reversions seen in a limited number of patients following transplantation
- The delayed time to rebound in the Mississippi baby
- The persistent immune activation in patients with HIV-RNA levels <50 copies/ml

Strategies directed toward “curing” HIV-1 need to include approaches designed to eliminate cells harboring such proviruses
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Colleen Ludka

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