Evidence of frequent HIV-1 superinfection in a cohort of Kenyan women

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More than 20 cases of HIV-1 superinfection have been reported:

- more commonly with viruses that are distantly related (intersubtype)
- few intrasubtype superinfection cases (mainly subtype B)

Initial population-based studies measuring incidence of superinfection, focused on cases with limited follow-up:

- superinfection was detected within ~ first year of initial infection, at an incidence approaching that of first infections. (Chohan B, et al., JV, 2005 and Smith DM, et al., JAMA, 2004).
The rationale of doing a larger study of the incidence of HIV-1 superinfection

- To determine how commonly HIV-1 superinfection occurs beyond one year of initial infection.

- To identify the frequency of superinfection with more closely related viruses (besides non-subtype B)
  - especially in heterosexual transmission.
Samples obtained from a prospective cohort of high risk women - Mombasa, Kenya (1993 - 2007)

Time of infection is determined by both serology and RNA testing

~ 1800 HIV-1 negative women enrolled with 300 seroconversions. HIV-1 seroincidence of ~ 8.3 cases/100 py
Characteristics of Mombasa FSW cohort

- These women continue to be exposed to new strains of HIV-1.
  They have an average of 1-2 sex partners per week, which is less than many sex worker cohorts.

- Long term follow-up, in some cases over a decade post initial infection.

- Three subtypes are common in this region:
  Subtype A ~ 80%
  Subtype D ~12%
  Subtype C ~ 7%
Analyzed 36 women within one year of initial infection who were infected with the most prevalent subtype (A) in Kenya.

With at least 5 yr follow-up and were ARV naïve - examine sequences at ~5 years PI.

Diagram:
- Plasma viral levels (RNA copies/ml)
- Diversity 1%/yr
- Evolution
- > 10%
- ~30%
- ~5%
- A
- A*D
- C
- <1 yr PI
- ~5 years post-infection
Methods and Results

- Phylogenetic analysis (maximum likelihood) and analyses divergence of viral sequences obtained from 36 women:
  - based on partial viral *gag* (670bp) and *envelope* (1.2kb) sequences,
  - obtained at two time-points;
    - early time point: range 17 - 338 DPI (median 111 DPI),
    - chronic infection: range 3.6 - 7.2 yrs (median 5.2 yrs PI)

- Suggests 7 possible cases of superinfection
Early and chronic viral sequences:
- cluster together suggesting single infection in most subjects;
- separate clustering suggests possible superinfection cases.
Examining two regions of the genome may increase the detection of superinfection, because recombination could mask cases of single infection.
The 7 possible superinfection cases were detected in either env and/or gag sequences.
One of possible case (QA413) of superinfection: Analysis of env sequences at initial and chronic time-points (DPI)

Days
Sequence
A
A*

Days 57 1346

estimated days since infection

A + 11
A* + 3

4.9 5.3 5.6 5.2 5.1 4.9 5.0 5.0 4.9

-200 0 200 400 600 800 1000 1200 1400 1600
Estimate time of superinfection: analysis of sequences and Allele-specific PCR from sequential time-points

Superinfection between 714 - 1007 days PI (midpt = 2.4 yrs PI)
Phylogenetic tree (ML) of \textit{env} sequences from all time points: case QA413

Early and chronic viral sequences:
- from all time-points cluster together with initial subtype A

Some viral sequences from 1007 days onwards:
- form a separate cluster of new subtype A (A*) viruses
- divergence of \sim 14% from initial subtype A sequence
Case of HIV-1 superinfection - QA413

- A case of **intrasubtype** superinfection
  - initially infected with subtype A and reinfected with another subtype A (A*).

- Superinfection occurred in the face of high viral load and during chronic infection ~ 2.4 yrs PI

- After superinfection, both subtype A viruses **coexisted** together through 3.7 yrs PI.
Summary - Superinfection in 7 cases occurred at different times after initial infection

From the 36 women - 7 women identified as possible superinfection cases in *gag* and/or *env* sequences

<table>
<thead>
<tr>
<th>ID</th>
<th>Estimated time to superinfection</th>
<th>Subtype (env)</th>
<th>Subtype (gag)</th>
<th>Type of reinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>initial chronic</td>
<td>initial chronic</td>
<td></td>
</tr>
<tr>
<td>QA413</td>
<td>~ 2.4 yrs</td>
<td>A</td>
<td>A + A*</td>
<td>A</td>
</tr>
<tr>
<td>QB685</td>
<td>~ 2.4 yrs</td>
<td>A</td>
<td>A</td>
<td>A*</td>
</tr>
<tr>
<td>QB045</td>
<td>~ 5.1 yrs</td>
<td>A2</td>
<td>A2 + A1</td>
<td>D/A2</td>
</tr>
<tr>
<td>QB726</td>
<td>~ 2.4 yrs</td>
<td>A</td>
<td>A + A*</td>
<td>D</td>
</tr>
<tr>
<td>QD022</td>
<td>~ 5.2 yrs</td>
<td>A</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>QB850</td>
<td>~ 2 months</td>
<td>A</td>
<td>A + A/D</td>
<td>A</td>
</tr>
<tr>
<td>QC885</td>
<td>~ 3.6 months</td>
<td>A</td>
<td>A</td>
<td>C</td>
</tr>
</tbody>
</table>
Conclusions (1)

- Incidence of HIV-1 superinfection is high in these high-risk women
  - the incidence of superinfection was ~ half the rate of initial incidence in cohort.

- Analyses of 2 regions of genome allowed detection of cases of superinfection - would have been missed if only one region were analyzed
  - may suggest even more cases would be detected if the whole genome was analyzed at multiple times PI.

- Superinfection may occur at different times after the initial infection
  - superinfection detected within a range of 2 months - 5 years: superinfection is not limited to a ‘window’ after initial infection.
Conclusions (2)

- Superinfection is not limited to viral strains that are distantly related; it can occur with closely related viruses
  - 3 out of 7 cases were intrasubtype superinfection.

- Superinfection can occur in the face of high viral replication and is not limited to cases where the first virus is of low replication fitness
  - both viruses can exist at similar levels for several years.
Implications of HIV-1 superinfection

The high frequency of HIV-1 superinfection suggests that it may be common

- suggesting that immune responses generated to natural HIV-1 infection may not be protective.

- additional studies examining immune responses in population-based cases of superinfection may provide insights into immune correlates that do not provide protection in HIV-1 infected individuals.
Divergence for each simulated superinfection

Gold: simulated superinfections (subtype A), $env = \text{range 10.4 - 34.2\%}$; $gag = \text{range 5.4 - 17.1\%}$

Purple: subjects
Strain-subtype specific PCR of PBMC DNA QA413

DPI 275  607  714  1007

Clones

A  A*  A

Uni

10 c 10 c 10 c 10 c

10^3 c 10^3 c 1 c 1 c