Cell Associated Transmission

Manish Sagar
HIV-1 TRANSMISSION

Index Case
CCR5 or CXCR4

Transmission

Primary Infection
CCR5

Homogeneous Clonal
40 – 80%

Heterogeneous Polyclonal
20 – 60%
**HIV acquisition versus exposure**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Relative Risk per 10,000 Exposures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Transfusion</td>
<td>9,000</td>
</tr>
<tr>
<td>Needle-sharing</td>
<td>67</td>
</tr>
<tr>
<td>Receptive penile-anal intercourse</td>
<td>50</td>
</tr>
<tr>
<td>Percutaneous needle stick</td>
<td>30</td>
</tr>
<tr>
<td>Receptive penile vaginal intercourse</td>
<td>10</td>
</tr>
<tr>
<td>Insertive penile anal intercourse</td>
<td>6.5</td>
</tr>
<tr>
<td>Insertive penile vaginal intercourse</td>
<td>5</td>
</tr>
<tr>
<td>Receptive penile oral intercourse</td>
<td>1</td>
</tr>
<tr>
<td>Insertive penile oral intercourse</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Powers et al., Lancet Infect. Dis. 2008
Ancestral strains are preferentially transmitted

A)

HIV Status by Year

<table>
<thead>
<tr>
<th>Year</th>
<th>Male Partner</th>
<th>Female Partner</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>+</td>
<td>n/a</td>
</tr>
<tr>
<td>1995</td>
<td>n/a</td>
<td>n/a</td>
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<tr>
<td>1996</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1997</td>
<td>+</td>
<td>n/a</td>
</tr>
<tr>
<td>1998</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>1999</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>2000</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>2001</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>2002</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

B)

C)

Slope = 0.0019
p < 0.05
n = 22

Redd et al., JID 2012
Transmitted viruses are more closely related to previously circulating strains

Redd et. al. JID 2012
Previously circulating strains are sensitive to contemporaneous autologous sera

Viruses present in newly infected subjects are highly sensitive to the transmitting partner antibodies

Richman et al., PNAS 2003
Derdeyn et al., Science 2004
How do newly infected individuals acquire strains that are both more closely related to previously circulating donor strains and likely more sensitive to antibodies present in the donor at the time of transmission?

- Hypothesis: Newly infected individuals may acquire HIV-1 from cell associated as opposed to cell-free virus.
Acquisition of multiple variants are likely linked events
Transmitted viruses

- 8 recipient donor pairs
- "Founder" virus most similar to donor virus found in blood (5 of 8 cases). In 3 of these 5 cases, source was a plasma virus.
- In the remaining cases, "founder" virus most closely related to a genital swab isolate (RNA) and in 1 case cell associated.

Boeras et. al., PNAS 2011
PBMC or plasma derived virus?

- Sampled 8 recipient donor pairs.
- All newly infected subjects were sampled during acute infections.
- Donor variant most closely related to recipient sequence from plasma in 3/8 cases and/or in PBMC samples in 6/8 cases.

Frange et al., Plos One 2013
Sequencing Studies

- Analysis of recipient donor pairs shows that plasma or PBMC donor virus is often most closely related to the founder virus in the newly infected partner.
- Lack of compartmentalization between plasma and PBMC donor virus make it difficult to conclusively demonstrate if acquired virus is from the plasma or PBMC.
Phenotypic studies

- Recipient viruses have a unique phenotype that confers fitness for transmission, potentially enhanced cell to cell transmission.

- Compare recipient and transmitter virus phenotypic properties.
## Cohort demographics

<table>
<thead>
<tr>
<th>Couple</th>
<th>Type</th>
<th>Int. Days $^2$</th>
<th>Partner Interval $^3$</th>
<th>Recipient CCR5 $^4$</th>
<th>Recipient CXCR4 $^5$</th>
<th>Recipient Tropism $^6$</th>
<th>Transmitter CCR5</th>
<th>Transmitter CXCR4</th>
<th>Transmitter Tropism</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>FTM</td>
<td>17</td>
<td>3</td>
<td>7.24</td>
<td>&lt;0.1</td>
<td>R5</td>
<td>8.27</td>
<td>&lt;0.1</td>
<td>R5</td>
</tr>
<tr>
<td>888</td>
<td>MTF</td>
<td>74</td>
<td>19</td>
<td>10.39</td>
<td>&lt;0.1</td>
<td>R5</td>
<td>11.91</td>
<td>&lt;0.1</td>
<td>R5</td>
</tr>
<tr>
<td>890</td>
<td>MTF</td>
<td>138</td>
<td>12</td>
<td>3.79</td>
<td>&lt;0.1</td>
<td>R5</td>
<td>2.27</td>
<td>&lt;0.1</td>
<td>R5</td>
</tr>
<tr>
<td>394</td>
<td>MTF</td>
<td>93</td>
<td>2</td>
<td>7.79</td>
<td>&lt;0.1</td>
<td>R5</td>
<td>10.85</td>
<td>&lt;0.1</td>
<td>R5</td>
</tr>
<tr>
<td>927</td>
<td>MTF</td>
<td>324</td>
<td>46</td>
<td>12.55</td>
<td>&lt;0.1</td>
<td>R5</td>
<td>13.37</td>
<td>&lt;0.1</td>
<td>R5</td>
</tr>
<tr>
<td>2769</td>
<td>MTF</td>
<td>149</td>
<td>46</td>
<td>5.69</td>
<td>&lt;0.1</td>
<td>R5</td>
<td>5.34</td>
<td>0.65</td>
<td>R5/X4</td>
</tr>
<tr>
<td>2810</td>
<td>MTF</td>
<td>161</td>
<td>23</td>
<td>5.49</td>
<td>&lt;0.1</td>
<td>R5</td>
<td>6.12</td>
<td>&lt;0.1</td>
<td>R5</td>
</tr>
<tr>
<td>SR-5</td>
<td>MTF</td>
<td>17</td>
<td>0</td>
<td>12.62</td>
<td>&lt;0.1</td>
<td>R5</td>
<td>9.72</td>
<td>&lt;0.1</td>
<td>R5</td>
</tr>
<tr>
<td>SR-20</td>
<td>MTF</td>
<td>91</td>
<td>34</td>
<td>6.70</td>
<td>&lt;0.1</td>
<td>R5</td>
<td>7.24</td>
<td>&lt;0.1</td>
<td>R5</td>
</tr>
</tbody>
</table>
Replication Competent Recombinant Viruses

- Full length envelope sequences amplified from each sample.
  Median 4 (range 4 – 8) independent PCRs

Yeast gap-repair

NL4-3 Sequences

293 T

PBMC

High titer replication competent virus

Etemad et al., JVI 2009
Dudley et al., Biotechniques 2009
Recipients and transmitters are virologically linked.
Sensitivity of receptor and fusion inhibitors

A. Recipient ○ Donor □

CD4 B4 MAb IC50 (ug/ml)  

B. Maraviroc IC50 (nM)  

C. T-20 IC50 (ug/ml)  

Estimated days post infection

D. Recipient/donor B4 MAb IC50  

ρ = -0.27  

p = 0.49

E. Recipient/donor maraviroc IC50  

ρ = 0.69  

p = 0.04

F. Recipient/donor T-20 IC50  

ρ = 0.28  

p = 0.47
Replication in CD4+ T cells

Graph showing RLU/ul vs. Days PI for R-1 and D-1.

Graph showing Recipient/Donor AUC vs. Couples.

Graph showing Recipient/donor AUC vs. Estimated days post infection.

Statistical values: $p = 0.32, p = 0.40, p = 0.03$.
Replication in MDDC-CD4+ T cell cocultures

- Replication in MDDC-CD4+ T cell cocultures: $p < 0.001$

- Recipient / Donor AUC
- Estimated days post infection

- $\rho = 0.08$  $p = 0.85$
- $\rho = 0.45$  $p = 0.23$
Replication in LC-CD4+ T cell cocultures

Estimated days post infection

Recipient /donor AUC

Replication in LC-CD4+ T cell cocultures

p = 0.02

p = 0.02

p = 0.78
Transmission and acute infection

Host

Vagina

Viral quasi species

RS

X4

CD4+ T cell

Macrophage

Afferent lymphatics

Efferent lymphatics

GALT

Lymph node

Thoracic duct

Spleen

Lung

Brain

Liver

Virus

Cervix

Crossing the mucosal barrier

Virus-host cell interactions and local propagation of infection

Dissemination to draining lymph nodes

Systemic dissemination of virus and infected cells

Establishment of lymphatic tissue reservoir

Innate and adaptive immune defenses

Draining lymph-node

Submucosa

Mucosal barrier

Pope et al., Nature Medicine 2003
Gut homing receptor, $\alpha_4\beta_7$
Gut homing receptor, α4β7

Incubation with RA (6 Days)

T Cells (CD8+ & CD4+):

1h@ 4°C
Wash
β-gal assay to monitor virus replication

CD4+

1h@ 4°C
Wash
qPCR to monitor virus binding

CD8+

RNA extraction

CD4+

CD8+
α4β7 usage

Recipient/Donor RNA copies

Estimated days post infection

Recipient/Donor AUC

Couples

p = 0.03

p = 0.60

p = 0.10

ρ = 0.36

p = 0.34

ρ = 0.60

p = 0.10

α4β7 usage

p = 0.03

p = 0.01

p = 0.01

p = 0.03
Transmitted founder versus chronic control viruses

- Full length molecular clones of phylogenetically estimated T/F viruses
- 13 Subtype B T/F versus 5 CC from 4 subjects
- 14 Subtype C T/F versus 9 CC from different subjects
- All T/F were unrelated to CC

Parrish et al., PNAS 2013
T/F more infectious, higher env content, enhanced DC-T cell transmission and greater replication in presence of IFN-α.
## Early/Transmitted versus Chronic/Donor

<table>
<thead>
<tr>
<th>Properties</th>
<th>Couples</th>
<th>T/F vs CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>Envelope</td>
<td>Full-length molecular clone</td>
</tr>
<tr>
<td>Sampling</td>
<td>Seronegative to 1 year</td>
<td>Phylogenetically estimated T/F</td>
</tr>
<tr>
<td>Comparison</td>
<td>Swarm present in transmitter</td>
<td>Unrelated chronic phase virus</td>
</tr>
<tr>
<td>Enhanced Infectivity</td>
<td>Transmitter</td>
<td>T/F</td>
</tr>
<tr>
<td>Higher replication in DC-T cells</td>
<td>Transmitter</td>
<td>T/F</td>
</tr>
<tr>
<td>α4β7 usage</td>
<td>Transmitter</td>
<td>No difference</td>
</tr>
<tr>
<td>CD4 usage</td>
<td>No significant difference</td>
<td></td>
</tr>
<tr>
<td>CCR5 usage</td>
<td>No significant difference</td>
<td></td>
</tr>
<tr>
<td>Fusion</td>
<td>No significant difference</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions

- Newly infected subjects may acquire cell associated virus.

- Cell associated and cell free virus are rarely compartmentalized making it difficult for sequence studies to determine origin of transmitted virus.

- Couple studies suggest that transmitter as compared to recipient swarm are more efficient in DC/LC T cell transfer.

- Because we did not examine T/F, it is possible that viruses with a transmission phenotype, such as cell associated replication, are selected against early after acquisition. In this case, variants with enhanced cell to cell replication must be enriched during the chronic phase of disease.

- Cell associated transmission may require different preventative strategies.
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