What makes the transmitted HIV-1 in newly infected patients unique from the donor inoculum?
What happens during primary infection?
Evidence of the genetic bottleneck at primary infection

Keele et al. PNAS 2009

Genotypic and phenotypic characterization of HIV-1 patients with primary infection
Zhu, et al.
Science 27 August 1993: 1179-1181.
Transmission Fitness

1. Is there anything unique about the acute HIV-1?
Replication efficiency in MDDCs and MDMs

King et al. 2013 JVI
Replicative fitness of acute versus chronic in PBMCs
Entry kinetics based of $T_{1/2}$ for T20

- Mean $T_{1/2}$ of 92.1 min for elite clones, 67.5 min for acute and 58.3 min for chronics
- This same difference in kinetics was observed in next subsequent step in replication, reverse transcription suggesting that this delay with elites reduces overall fitness

Lassen et al. 2009 PLoS Pathogens
Entry efficiency based on Affinofile

A

Add equal amounts of virus → High CCR5 → Low CCR5 → measure virus production

Decreasing CD4 levels

293T cell cultures

B

High CCR5

C

Low CCR5

% infection relative to max CD4/CCR5 vs Copies CD4/cell

% infection relative to max CD4/CCR5 vs Copies CD4/cell
Transmission Fitness

2. Establish a model for transmission fitness by multivirus infections of penile, cervical, vaginal, and rectal human tissue
New methodology to determine “transmission” fitness

Imperial College
Robin Shattock
Asna Siddiqui

CWRU
Immaculate Nankya

[Diagram of the methodology]
New methodology to determine “transmission” fitness

Lyse tissue, migratory cells, and co-cults
Harvest DNA/RNA

PCR amplify C2-V3 env complex HIV-1 mixtures

barcode

re-amplify tissue with “a” primer set
migratory cells with “b” primer set
co-cult cells with “c” primer set

combine equivalent amount of 300-400 barcoded amplified products

University of Manchester
John Archer
David Robertson

CWRU
Gabriele Nickel
Melanie Fleming
Ken Henry
New methodology to determine “transmission” fitness

1. collect migratory cells at 48h, incubate with PM1 cells for 10 days

2. lyse tissue, migratory cells, and co-cults

3. harvest DNA/RNA

4. PCR amplify C2-V3 env HIV-1 mixtures

5. combine equivalent amount of 300-400 barcoded amplified products

6. 454 FLX titanium sequence run

7. combine equivalent amount of 300-400 barcoded amplified products

8. PIPELINE FOR COMPETITION DATA ANALYSES

9. 1. sort/bin sequences based on barcode

10. 2. align sequences within a barcode bin

   - tissue
   - migratory cells
   - co-cult cells

<table>
<thead>
<tr>
<th>barcode</th>
<th>tissue</th>
<th>migratory cells</th>
<th>co-cult cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>6%</td>
<td>7%</td>
<td>1%</td>
</tr>
<tr>
<td>B2</td>
<td>17%</td>
<td>11%</td>
<td>9%</td>
</tr>
<tr>
<td>B3</td>
<td>32%</td>
<td>24%</td>
<td>26%</td>
</tr>
<tr>
<td>B4</td>
<td>11%</td>
<td>9%</td>
<td>5%</td>
</tr>
<tr>
<td>B5</td>
<td>8%</td>
<td>13%</td>
<td>19%</td>
</tr>
<tr>
<td>Cq0</td>
<td>&lt;.5%</td>
<td>&lt;.03%</td>
<td>&lt;.01%</td>
</tr>
<tr>
<td>Ck44</td>
<td>18%</td>
<td>22%</td>
<td>14%</td>
</tr>
<tr>
<td>Ci10</td>
<td>9%</td>
<td>12%</td>
<td>24%</td>
</tr>
</tbody>
</table>
Transmission Fitness

3. Compare the transmission fitness of acute versus chronic HIV-1
Acute versus chronic HIV-1 competitions

[Graphs showing percent replication in tissue and in tissue MC + PM1]
Summary of multivirus competitions

A. Relative replication in penile tissue

B. Relative replication in penile MC co-cultured with PM1 cells
Summary of multivirus competitions

- Average relative replication in tissue
- Average relative replication in tissue MC+ T cells (PM1)

Percentage of specific virus in accumulative multivirus competitions

- CHRONIC VIRUSES
  - I10
  - K44
  - Q0

- ACUTE VIRUSES
  - B1
  - B2
  - B3
  - B4
  - B7
  - B8
  - B9
  - B14
  - B17
  - B19
  - B20
DC-SIGN and Langerin: Capturing virus for transmission
Why are acute HIV-1 transmitted through genital mucosal better?

Imperial College
Robin Shattock
Daniel Stieh
How many N linked glycosylation sites?

| TF-HIV-1 gp120 sequences | 97 | 139 | 151 | 169 | 199 | 203 | 246 | 268 | 305 | 312 | 333 | 347 | 360 | 366 | 372 | 409-411 | 416 | 433-435 | 443 | 467 | 473 | 486 | 497 | 541 | 547 | 560 |
|--------------------------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------|-----|--------|-----|-----|-----|-----|-----|-----|-----|-----|
| B8                       | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B19                      | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B7                       | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B18                      | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B4                       | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B9                       | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B17                      | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B14                      | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B20                      | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B5                       | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B1                        | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B10                      | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B2                        | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B13                       | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B6                        | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B11                       | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| B3                        | N  | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | N   | Y     | N   | N      | N   | N   | N   | N   | N   | N   | N   | N   | N   |
| **domains**               |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **C1**                    |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **C1 V1/V2**              |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **C2**                    |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **C2 V3**                 |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **C3**                    |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **C3 V4**                 |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **C4**                    |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **C4 V5**                 |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **C5**                    |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **Total sites**           |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **TF-HIV**                |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **gp41**                  |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| **Consensus**             |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

**Case Western Reserve University**

**Center for AIDS Research**

**CFAIR**

**University of Pennsylvania**
Coming back to the in vivo analyses?
Do lectins (mannose binding proteins) trap virus in the mucosa?

- Extracellular mannose binding lectins/proteins (MBL/Ps)\(^1\)-\(^5\) and C-type lectins on epithelial cells\(^6\)-\(^8\) bind and trap pathogens with foreign high mannose, branched oligosaccharides.
- Mannose receptors on vaginal epithelial cells are thought limit pathogen infections (e.g. Chlamydia\(^9\)-\(^11\)) and may even induce MBPs upon direct pathogen binding\(^7\).
- Homozygote carriers for a variant MBL allele that decreases MBL concentrations (nearly 1000-fold in sera) were at high risk for HIV infection whereas heterozygotes showed slower disease progression\(^1\).
- In human foreskin, immunostaining of C-type lectins on resident LCs and DCs was masked by the overabundance of lectins on the surface of the stratified epithelial layers\(^4\).

Newly infected HIV-1 women were enrolled from a cohort of 4,439 women from family planning sites from Uganda and Zimbabwe evaluating the effect of hormonal contraception on HIV-1 acquisition (quarterly HIV-1 testing).

>100 Ugandan and >180 Zimbabwean women were recruited within 3 months of infection.

Morrison et al. AIDS 2010; Morrison et al. J.AIDS 2011
What we have found at primary infection in the endocervix versus the blood

A.  

- P3m = plasma sample at 3 mo.
- EC3m = endocervical sample at 3 mo.
- n = number of sequences
- 17U = patient 17 from Uganda

1 substitution from root per ring

endocervix

17U

n=1108

0.035

0.01 s/nt

B. V3 genetic diversity in pt 17U
(n = ~15 sequences/sample)

![Bar graph showing average genetic distance (s/nt) for 3 mo, 1 yr, 3 mo, 1 yr]

- plasma
- genital tract

C. Average genetic distance (s/nt)

- Ug plasma 3 mo
- Ug cervix 3 mo
- Zim plasma 3 mo

Gabriele Nickel,
Immaculate Nankya,
Taina Immonen
Conclusions

• HIV-1 genetic diversity may be higher at the site of infection (e.g. vaginal tract) than in the plasma at acute/early infection

• In our ex vivo models, chronic HIV-1 isolates appear to be “trapped and replicating” in the mucosal tissue whereas acute HIV-1 pass through the mucosal tissue via LC/DCs and transmit to T cells

• C-type lectin bind to high mannose sugars common to many pathogens but the high expression of these lectins on epithelial cells is often overlooked in favor of the “collecting” lectins on DCs or LCs. Maybe the mucosa is designed to keep pathogens out…

• Exposure of macaques with a mixed SHIVenv population based on 17 acute Env’s results in a single strain SHIVenv infection. This SHIVenv B3 had the lowest number of N linked sites and the lowest C-type lectin binding affinity
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