Transmitted HIV-1 is an R5 T-cell tropic virus while macrophage-tropic viruses are evolutionary dead ends with the ability to use low levels of CD4

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Sample population

Individuals from Malawi and South Africa heterosexually infected with Subtype C HIV-1

Analyzed entry phenotypes of env clones generated from:

• 34 acutely infected subjects = transmitted / founder viruses

• 32 chronically infected subjects = chronic control viruses
Methods that have led to the mischaracterization of viruses as macrophage-tropic

1. Definitional / dogma

T cell-tropic HIV = CXCR4 using

Macrophage-tropic HIV = CCR5 using
Methods that have led to the mischaracterization of viruses as macrophage-tropic

1. Definitional / dogma

T cell-tropic HIV = CXCR4 using

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2. Experimental

Monocyte derived macrophage (MDMs) differ greatly in their ability to support HIV replication. Most viruses are capable of some replication in macrophage.
We need a new method for assessing macrophage-tropism!
Macrophage tropism and CD4 usage

![Graphs showing infectivity (x 1,000 RLU) for different donors.](image-url)
Differential expression of CD4 has a **HUGE** effect on how susceptible macrophage are to infection.

Macrophage tropism and CD4 usage

**Graphs showing infectivity**

- **Donor 2** (15k CD4/cell)
- **Donor 3** (17k CD4/cell)
- **Donor 4** (14k CD4/cell)
- **Donor 5** (16k CD4/cell)
Macrophage tropism and CD4 usage

Differential expression of CD4 has a **HUGE** effect on how susceptible macrophage are to infection

\[ r^2 = 0.995 \]
Macrophage tropism and CD4 usage

Differential expression of CD4 has a **HUGE** effect on how susceptible macrophage are to infection

\[
\begin{align*}
\text{Relative infectivity} & \\
\text{Mean CD4 receptors per cell} & \text{Donor 2} \quad \text{Donor 3} \quad \text{Donor 4} \quad \text{Donor 5}
\end{align*}
\]

\[r^2 = 0.995\]
Affinofile Cells: Inducible CD4

Macrophage tropism and CD4 usage

Titration of CD4 Density in Affinofile Cells

CD4 Molecules per Cell vs. Doxy (ng/ml)
Macrophage tropism and CD4 usage

A: moderate to no decay - Macrophage tropic virus in CSF

B: rapid decay - T cell tropic virus in CSF
Macrophage tropism and CD4 usage

Infection of Affinofile cells is an accurate assay for macrophage tropism

A: moderate to no decay - Macrophage tropic virus in CSF

B: rapid decay - T cell tropic virus in CSF
Macrophage tropism and CD4 usage

Affinofile cells
Benhur Lee, UCLA

Percent infection

CD4 receptors / cell (x1000)
Transmitted/founder viruses are not macrophage-tropic and do not differ from chronically-derived viruses in their CD4 usage.
CCR5 usage

Affinoile cells
Benhur Lee, UCLA

Percent infection

Maraviroc concentration μM

Acutes
Chronics
CCR5 usage

High CCR5

- Sensitive
- Maraviroc Resistant

p-value = 0.01
Transmitted viruses are more likely to utilizing the maraviroc sensitive conformation of CCR5
Transmitted viruses are under-glycosylated relative to the viruses found in chronically infected subjects.
Higher glycosylation is correlated with a maraviroc resistant form of CCR5.
Conclusions

In heterosexual transmission in the setting of Subtype C HIV-1 the transmitted virus:

1. Requires high levels of CD4 to enter cells identifying T cells as its target cell
Potential target cells in the genital mucosa

- T cells: 450 CD4 ABS/μm²
- Macrophage: 20 CD4 ABS/μm²
- Dendritic cells: 15 CD4 ABS/μm²
Potential target cells in the genital mucosa:

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CD4 levels are too low.
Conclusions

In heterosexual transmission in the setting of Subtype C HIV-1 the transmitted virus:

1. Requires high levels of CD4 to enter cells identifying T cells as its target cell

2. Is enriched relative to chronic viruses to use a maraviroc sensitive conformation of CCR5

3. Has reduced glycosylation which is associated with the ability to use a conformation of CCR5 that is sensitive to maraviroc

4. Macrophage-tropic viruses are rare and are evolutionary dead-ends in each host where they evolve
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The transmitted virus is R5 T cell-tropic!