Infant Variants Require More CD4 for Entry than Maternal Variants Regardless of Transmission Route

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BACKGROUND

SUBJECTS

- Despite high level and persistent exposure to virus, the majority of children born to chronically HIV-infected women escape infection.
- Most infections that do occur are initiated by a single genetic variant selected from a diverse quasi-species swarm.
- Placental macrophages (Haufbaur cells), the HIV-susceptible fetal cell population in closest proximity to the maternal circulation, express relatively low levels of CD4.
- The developing fetal and infant immune system is biased towards immunologic tolerance and usually has a lower frequency of classically HIV-susceptible activated CD4+ T-cells.
- We therefore sought to determine the minimum level of CD4 expression required for entry by early-transmitted isolates compared to the maternal quasi-species (from at or near the time of transmission), and whether there was evidence for selective transmission of variants particularly adept at utilizing low levels of CD4.

METHODS

SUBJECTS

- All subjects were part of the Zambia Exclusive Breastfeeding Study (ZEBS), HIV positive, and ARV naïve at study enrollment.
- Seventeen transmission pairs were infected with subtype C virus (10 breast milk and 7 in utero transmitters).
- One transmission pair was infected with subtype G virus (a breast milk transmitter).
- ARV exposure at time of sampling was limited to peri-partum single-dose nevirapine as per Zambian government guidelines at the time.

ENVELOPE CLONING

- Full-length *env* genes were cloned from cell-free plasma or genomic DNA following nested PCR.
- Input cDNA/DNA was subjected to limiting dilution and in most cases only one clone was selected per PCR reaction.
- *env genes* were sequenced bi-directionally and screened for evidence of inter-patient or reference strain contamination, template re-sampling, and APOBEC-mediated hypermutation.
- A total of 158 unique envelopes from 18 mother-infant transmission pairs were included in the study.

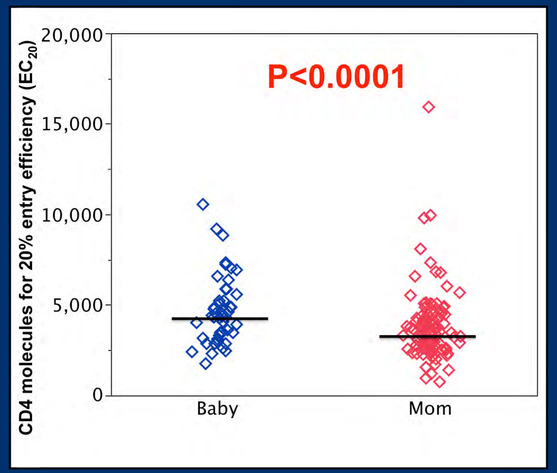
AFFINOFILE ASSAY

- Pseudotype viruses were produced by co-transfection of 293T/17 packaging cells with an expression plasmid bearing the envelope of interest and an *env*-deficient backbone.
- Affinofile cells were induced to maximize CCR5 expression while varying CD4 across an ~2 $\log_{10}$ range. After 24 hour incubation, ~2000 IU of virus (MOI ≤0.1) was added. CD4 and CCR5 concentrations were quantified at the time of infection using the BD QuantiBRITE™ system.
- Infection was quantified by luciferase activity read at 48 hours after virus addition using the NEB Gaussia luciferase kit and an Optima™ luminometer.

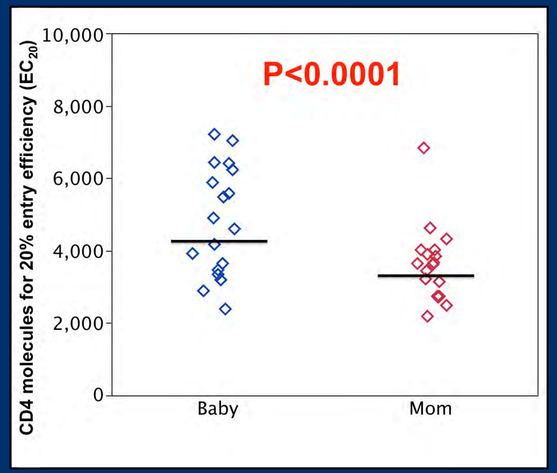
ANALYSIS

- Luciferase activity was normalized as a percentage of entry under saturating CD4 conditions and pooled across a minimum of 3 independent experiments. Percent entry was then plotted by absolute number of CD4 molecules/cell and a logarithmic curve fit was performed (y=m*ln(x)+b). Median R^2 for curve-fits was 0.82.
- Infection curves were used to calculate the number of CD4 molecules/cell necessary to achieve a pre-specified 20% of maximal entry (EC_{20}) for each viral variant.
- EC_{20}s for epidemiologically linked mother-infant pairs were compared using the Generalized Estimating Equation (GEE) with an unstructured correlation matrix.

Infant Isolates Require More CD4 for Entry than Epidemiologically-Linked Maternal Isolates

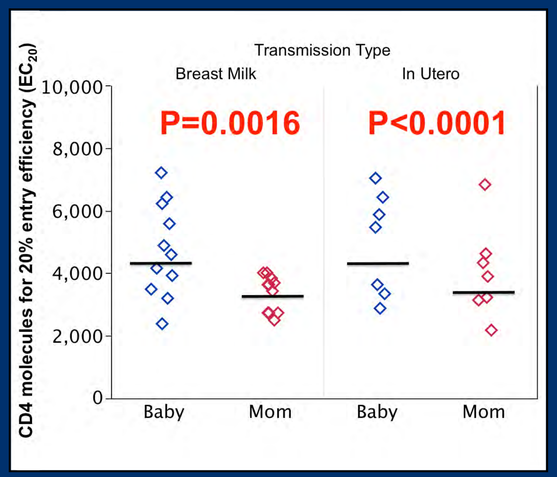


All 158 clones from 18 pairs

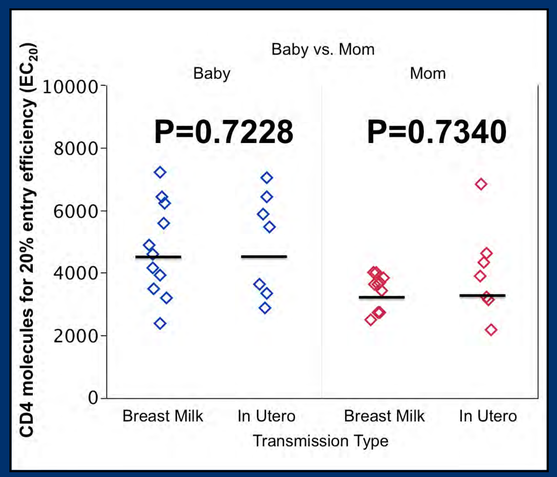


Per-pair median EC_{20}

Elevated CD4 Requirement is Not Route-Specific

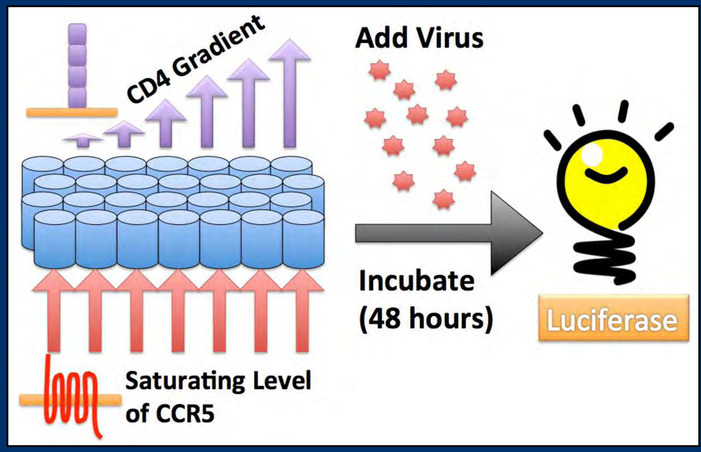


Median EC_{20}s by transmission type (11 breast milk, 7 in utero)

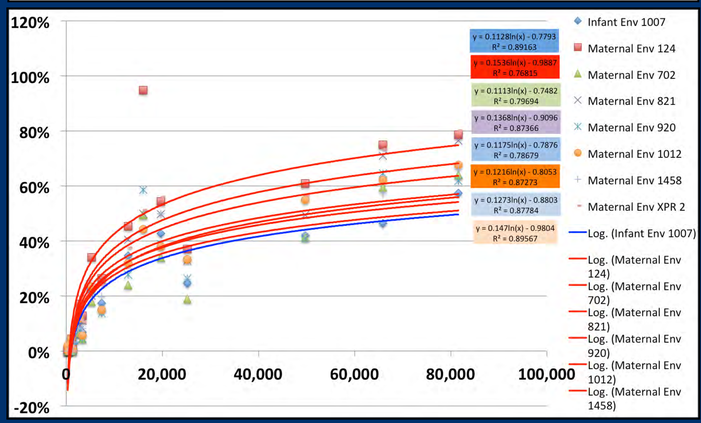


Comparison of inter-group median EC_{20}s (i.e. mom vs. mom by route)

Affinofile Assay Overview



Exemplar Log-Linear Plot for Single Subject



CONCLUSIONS

- Our data indicate that vertical transmission of HIV does not select for highly efficient usage of CD4, and may require relatively high CD4 levels for initiation and/or propagation of infection.
- Given the lack of route specificity, the described selection is probably not occurring at the site of initial barrier breach (e.g. placenta or gut epithelium), and may represent a common pathway of amplification/outgrowth following the initial seeding event.
- Our data are consistent with a role for transient inflammation in enhancing susceptibility to infection.

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