Poster 329

# Transient Compartmentalization of SIV Variants in the Breast Milk of African Green Monkeys

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## Background

In contrast to HIV-infected humans and SIV-infected rhesus monkeys, natural hosts of SIV, African green monkeys (AGMs), rarely transmit the virus postnatally. Previous studies in humans and rhesus monkeys revealed that breast milk populations of humans and rhesus monkeys are not genetically distinct from those in plasma. As the breast milk virus population of natural hosts of SIV remains uncharacterized, we sought to examine the genetic and functional diversity of breast milk SIV variants in this limited transmission setting.

## Methods

- **Animals, infection, and sample collection:** We intravenously infected six female hormone-induced lactating AGMs with the infectious molecular clone T/F SIV variant (SIVsab92018ivTF). Plasma and breast milk samples were collected at 5 months and 1 year post-infection.
- **Single genome amplification and sequencing of the *envelope* gene:** Viral RNA was extracted from the plasma and breast milk samples. cDNA was synthesized and endpoint diluted to a concentration that yielded no more than 30% positive wells and conforms to a Poisson distribution of a single template per reaction.
- **Cloning and Env pseudovirus production:** plasma and milk env genes from one monkey were cloned and used to produce Env pseudoviruses to assess for infectivity and neutralization sensitivity
- **Phylogenetic Sequence Analysis**: Sequences were aligned using MUSCLE, and maximum likelihood trees of plasma and milk virus *env* sequences were constructed using PAUP*. Pairwise distances and branch lengths were calculated using PAUP*. Mutations were visualized, and potential N-linked glycosylation sites were enumerated using Los Alamos' sequence analysis tools.
- **Statistical Analysis**: Compartmentalization was assessed using the Slatkin-Maddison test implemented by HyPhy software. The proportion of genetically identical sequences in plasma and breast milk were compared using Fisher's exact test.

## Results

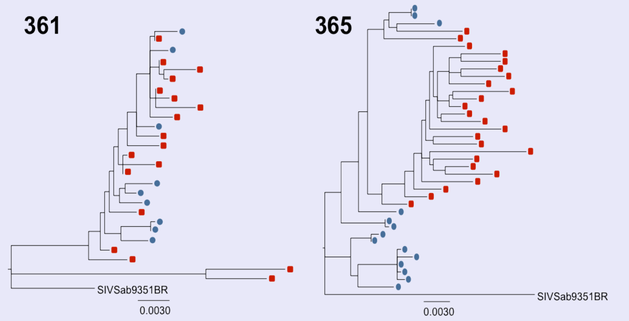



**Figure 1. Maximum likelihood trees of plasma and breast milk virus variants of AGMs three years after infection with an uncloned SIVsab virus stock.** The trees show full-length SIV *env* sequences amplified by single genome amplification from plasma (red squares) and milk (blue circles). There is evidence of compartmentalization between breast milk and plasma virus variants. The p-values were generated by the Slatkin-Maddison test of compartmentalization. The scale bar represents 0.003 substitutions per site.

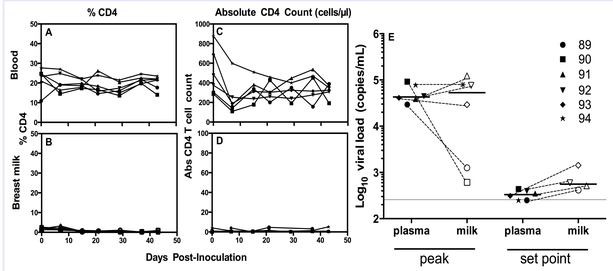



**Figure 2. CD4+ T cell counts and viral load in blood and breast milk of AGMs acutely infected with a T/F SIVsab variant.** The percentage of CD4+ T lymphocytes in six AGMs in blood (A) and breast milk (B) during acute infection with SIVsab92018ivTF. The absolute CD4 T cell count in blood (C) and breast milk (D) during acute infection with SIVSab92018ivTF. The peak SIV viral load in plasma (closed symbol) is similar to that in breast milk (open symbol) of four SIV-infected AGMs ($p = 0.93$), whereas the set point milk viral load trends towards a magnitude higher than that in plasma ($p = 0.09$) (E). Dashed lines are drawn between plasma and milk viral loads from the same monkey, and solid lines display the medians of each data set. Dotted line represents the minimum of detection in the virus load assay.

**Table 1.** Number of clonally amplified virus variants in plasma and breast milk virus variants of SIV-infected AGMs.

| Monkey | Sample Type | No. of amplicons that are part of a group of identical sequences | Total No. of Env sequences | Percentage of Env sequences that are part of a group of identical sequences | P-value from Fisher's Exact Test |
|---|---|---|---|---|---|
| 89 | Plasma | 13 | 19 | 68.4 | .1274 |
| | Breast milk | 18 | 20 | 90.0 | |
| 91 | Plasma | 2 | 18 | 11.1 | <.0001 |
| | Breast milk | 15 | 17 | 88.2 | |
| 92 | Plasma | 7 | 19 | 36.8 | <.0001 |
| | Breast milk | 18 | 18 | 100 | |
| 93 | Plasma | 2 | 17 | 11.8 | .2245 |
| | Breast milk | 5 | 16 | 31.3 | |
| 94 | Plasma | 2 | 18 | 11.1 | 1 |
| | Breast milk | 0 | 8 | 0 | |

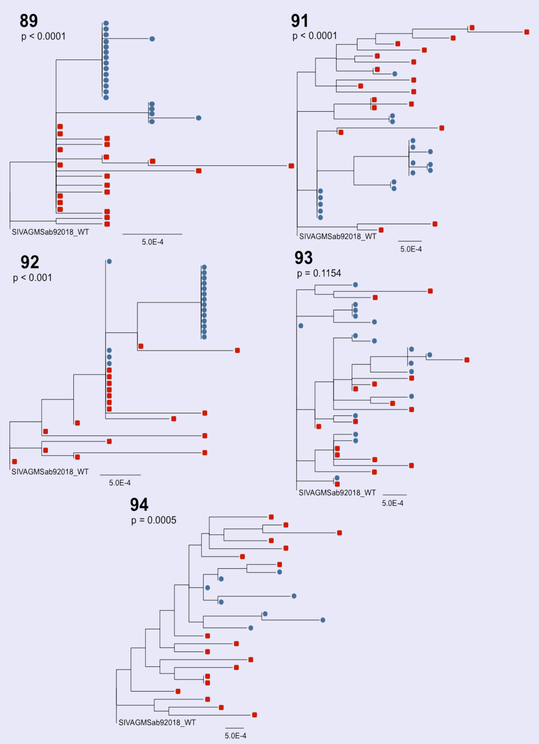



**Figure 3. Compartmentalization of SIVsab92018ivTF *env* variants in breast milk of four of five AGMs at five months post-infection.** Maximum-likelihood trees of full-length SIV *env* sequences amplified by single genome amplification from plasma (red squares) and milk (blue circles). The p-values were generated by the Slatkin-Maddison test of compartmentalization. The scale bar represents .0005 nuclear substitutions per site.

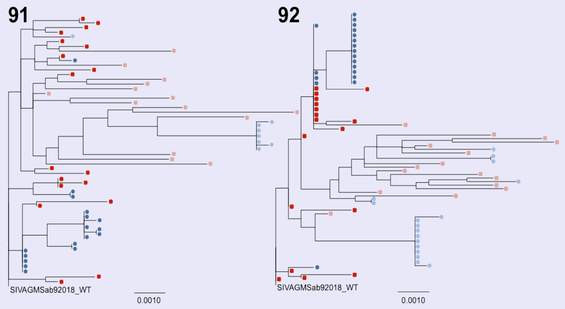



**Figure 4. Maximum likelihood trees of plasma and breast milk virus variants from five months post-infection and one year post-infection.** Trees show full-length SIV *env* sequences from plasma (red squares) and milk (blue circles) at five months post-infection and plasma (pink squares) and milk (light blue circles) at one year post-infection. There is transient compartmentalization of virus variants in breast milk of SIV-infected AGMs but milk virus variants do not evolve distinctly from that in plasma over time. There are large groups of identical or nearly identical sequences among the breast milk virus variants. The scale bar represents .001 nuclear substitutions per site.

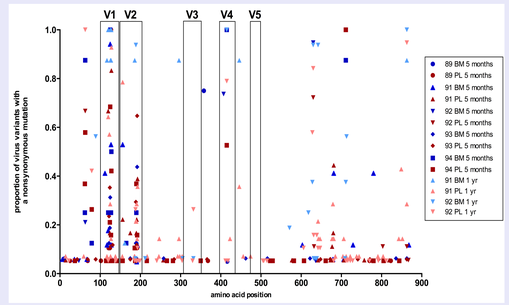



**Figure 5. Nonsynonymous amino acid substitutions in milk and plasma Env** Env that is distinct from those in plasma across the five monkeys. High frequency substitutions in both plasma and milk virus variants are clustered in the V1 and V2 loop regions. **variants of SIV-infected AGMs.** The frequency of substitutions in plasma virus variants and breast milk virus variants at each amino acid position in the SIVsab92018ivTF Env is shown. There is minimal clustering of substitutions in breast milk virus variants to a specific region of the

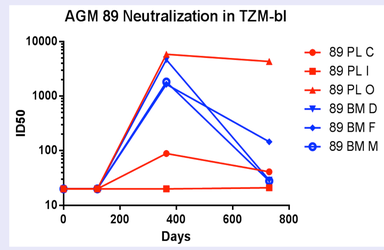



**Figure 6. Comparison of the autologous plasma neutralization sensitivity of SIV Env variants isolated from breast milk and blood of monkey 89.** Autologous plasma neutralization, expressed as inhibitory dose 50% (ID50), during acute and chronic infection of plasma and breast milk SIVsab92018 Env pseudovirus variants obtained 5 months post-infection. Breast milk variants are in blue and plasma viruses are in red.

## Conclusions

- Compartmentalization of low diversity breast milk *env* variants in AGMs is transient and may be a result of low number of CD4+ T lymphocyte target cells in the breast milk compartment of AGMs
- This is in contrast with the limited compartmentalization previously observed in humans and rhesus monkeys
- As there is a genetic bottleneck in postnatal virus transmission, the restricted diversity of breast milk virus variants and the limited number of CCR5-expressing CD4+ target cells in infant AGMs may together contribute to the rarity of postnatal transmission in natural hosts.

## Acknowledgements

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## References

Ho, C et al, Transient Compartmentalization of Simian Immunodeficiency Virus Variants in the Breast Milk of African Green Monkeys. *Journal of Virology*. 2013