Intact proviruses from lymph nodes are a likely source of viral rebound in SHIV-infected NHP

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BACKGROUND

Hypothesis: A comparative analysis of the reservoir persisting in different compartments can inform on the anatomical origin of the viral rebound upon antiretroviral treatment interruption (ATI).

The persistence of SIV/HIV reservoirs is the main obstacle to a cure and the cause of viral rebound after ART cessation. However, the anatomical source of viral rebound remains elusive. We characterized the proviral landscape in various tissues of virally suppressed SHIV-infected Rhesus Macaques before ART interruption (ATI) and matched it with the *env* sequences from plasma viruses during rebound.

Lymph nodes harbor a high proportion of intact SHIV proviruses during ART and contribute to viremia upon ATI.

METHODS

We used a highly processive and accurate polymerase and PacBio sequencing to obtain near full-length (NFL) genome sequences from a cohort of 5 SHIV-infected Rhesus Macaques. Intact sequences were identified by an algorithm we designed. Single copy sequencing was used to obtain *Env* sequences from plasma rebound viruses 4 weeks after ATI.

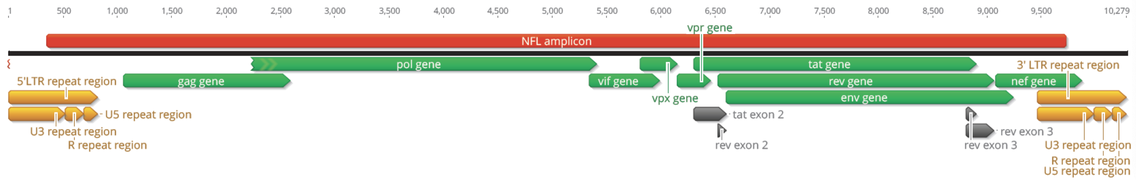


Figure 1. SHIV amplicons obtained by NFL sequencing. Red bar represents the portion of the SHIV genome covered by the amplification protocol.

RESULTS

Study design and reservoir quantification

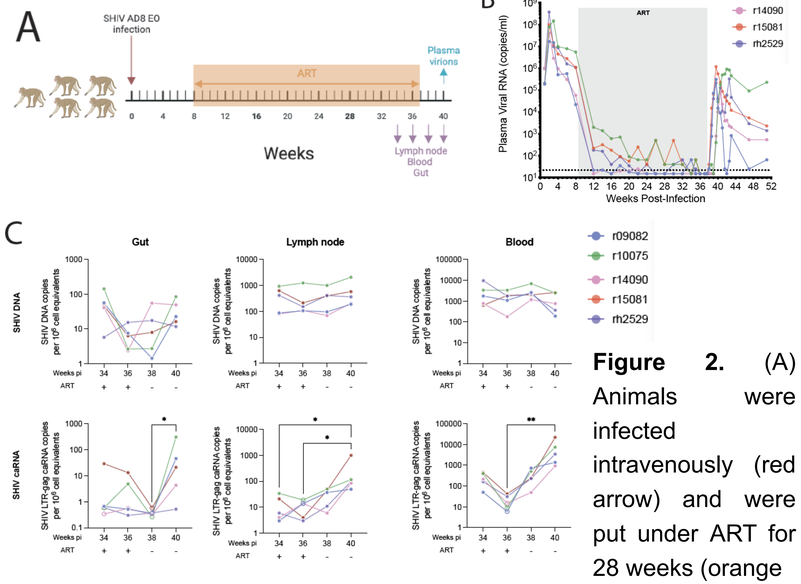
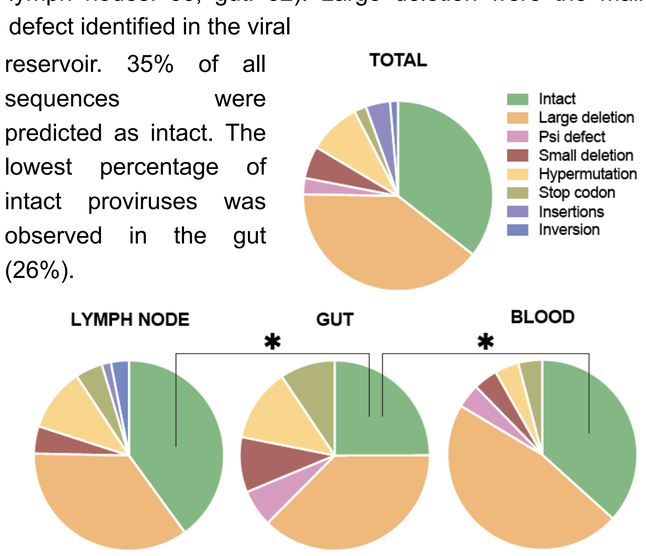


Figure 2. (A) Animals were infected intravenously (red arrow) and were put under ART for 28 weeks (orange area). Violet arrows indicate blood, lymph nodes and gut-sampling time points. Plasma virions *env* sequences were obtained from blood at week 40 (blue arrow). (B) Plasma viral load were undetectable in all animals at week 36, and all rebounded upon ATI. (C) LTR-Gag SHIV DNA copies remained relatively stable over time in all compartments. LTR-Gag cell-associated RNA copies increased significantly after ART interruption in all compartments. Overall, these results indicate that the virus rebounded and disseminated rapidly after ATI.

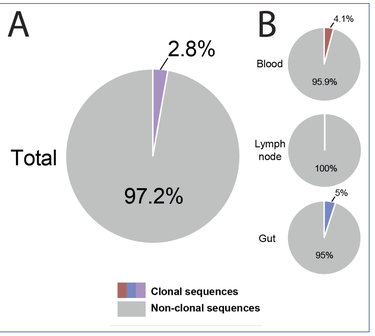
Integrity analysis

Figure 3. 144 proviral sequences were obtained (blood: 49; lymph nodes: 63; gut: 32). Large deletion were the main defect identified in the viral reservoir. 35% of all sequences were predicted as intact. The lowest percentage of intact proviruses was observed in the gut (26%).



Reservoir clonality

Figure 4. Pie chart representing the proportion of clonal sequences in the SHIV reservoir. From all sequences obtained clonal sequences represented 2.8% (A). Identical sequences were found exclusively in the blood and the gut (B).



Source of viral rebound

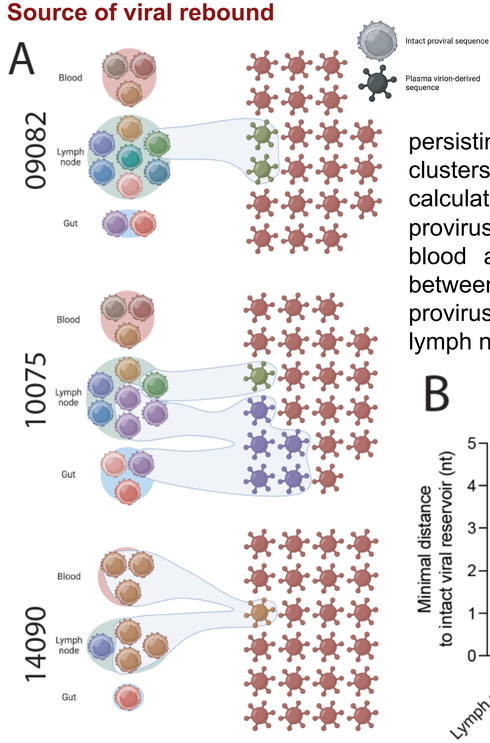


Figure 5. (A) Four different clusters of matched sequences were identified between *env* sequences from plasma rebounding virions and intact proviruses. Proviruses persisting in lymph nodes were identified in in all 4 clusters. (B) As shown by the minimal distance calculation, rebound viruses were genetically closer to proviruses persisting in lymph nodes compared to blood and gut. (C) The median genetic distance between individual rebounding viruses and intact proviruses from each compartment was lower in lymph node and blood compared to gut.

CONCLUSIONS

The SHIV reservoir is largely composed of unique proviral sequences, suggesting that cell proliferation has a minor contribution to viral persistence in this model. Our results further suggest that intact proviruses persisting in the lymph nodes may be a preferential source of viral rebound upon ATI.