HIV Drug Resistance Program
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Pol Versus Env Genetics in SHIV-Infected Macaques Highlights Importance of Phylogenetic Signal

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BACKGROUND

Previously we investigated HIV *pol* populations in RT-SHIV-infected macaques by single-genome sequencing to determine if low-level replication was a source of residual viremia during ART and to investigate viral compartmentalization across tissues. Using this approach, we found no evidence for evolution during suppressive ART and little evidence of viral compartmentalization. To investigate the possibility that the low diversity in *pol* masked the emergence of new viral variants and/or compartmentalization, we applied the same methods to the more diverse *env* gene in the infected macaques.

METHODS

Two macaques (M03250 and K02396) received 20 weeks of ART (TNF, FTC, EFV) and one macaque (6760) was untreated. Longitudinal plasma samples (N=11) from treated macaques were analyzed by single-genome sequencing of a 1 kb pol fragment and a 2.5 kb *env* fragment. In the untreated animal 6760, tissues were collected at necropsy after infection for 30 weeks of infection and single-genome sequences were obtained from PBMCs, lymph nodes, spleen, thymus, gut tissues, bone marrow, and lung. The entire 2.5kb env fragment and the 101 nucleotide V3 region alone were evaluated separately for population diversity, divergence, and compartmentalization using phylogenetic and panmixia analyses, and compared to results from *pol*.

CONCLUSIONS

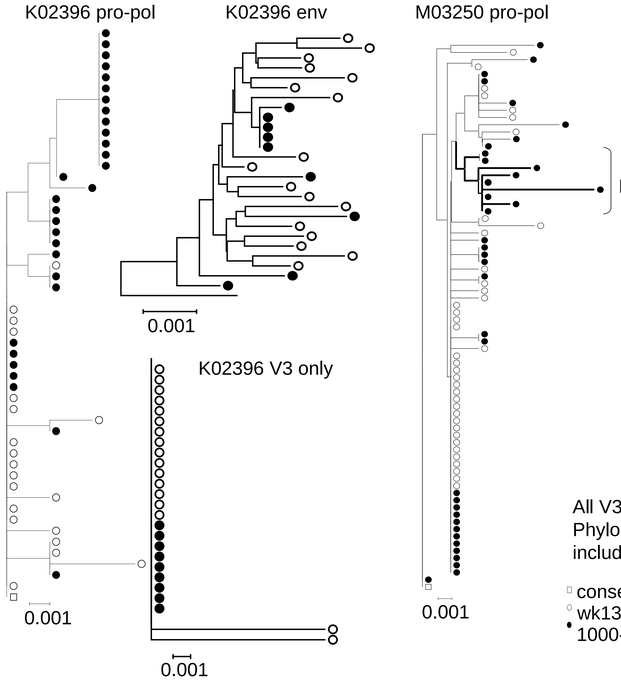
Comparisons of population genetics of *pol* and *env* in SHIV-infected macaques show that both regions have sufficient phylogenetic signal to assess evolution during ART and compartmentalization of virus populations. Although commonly used for genetic analyses, the V3 loop is under strong purifying selection that restricts its diversity and severely limits its usefulness for intra-individual analyses of viral genetics. These findings highlight the importance of performing single-genome and deep sequencing on regions of the viral genome with strong phylogenetic signal.

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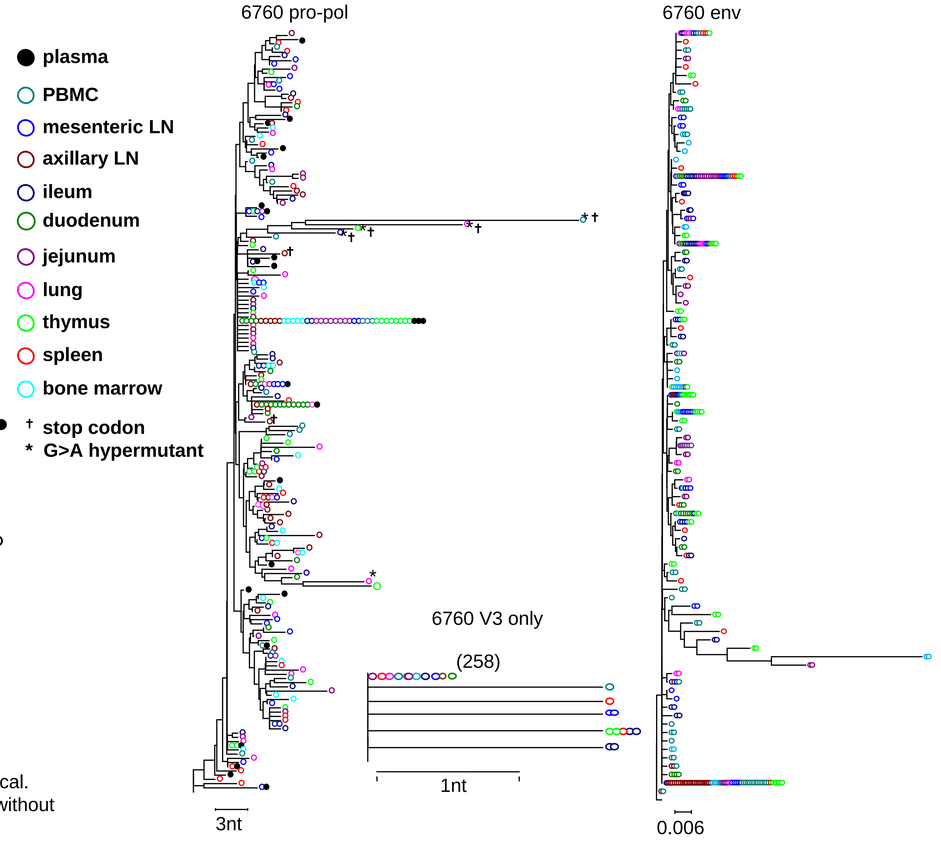
RESULTS

Previously we found diverse variants in *pol* but no new variants emerged in the plasma during suppressive ART, and viral population differences across tissues were due to varying frequencies of the same viral variants. Similarly, new phylogenetic and panmixia analyses of 2.5kb *env* sequences in plasma did not reveal the emergence of new variants during ART without drug resistance, showing that the lack of evolution in *pol* was not due to low phylogenetic signal in this region. E*nv* populations analyzed in tissues from 6760 were highly diverse but showed similar population structures to *pol* and a lack of tissue compartmentalization. By contrast, phylogenetic analyses of only the V3 *env* region showed very weak phylogenetic signals and little diversity, indicating that the V3 region is not appropriate to evaluate intra-individual populations for diversity, evolution, and phylogenetic structure.



All V3 sequences in M03250 are identical. Phylogenetic signal is completely lost without including conversed region in env.

- consensus wk1
- wk13
- 1000-10,000-fold VL decline (wk 17.5-37)



Animal 6760: Phylogenetic relationships between single-genome proviral sequences obtained from various anatomical compartments after thirty weeks of infection in untreated animal (6760). Sequences were analyzed at *pol*, *env*, and the *V3* loop.