Genetic and Age Distribution of Latent HIV Sequences in CD4+ T cell Subsets

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Poster #0342
CROI 2019
Seattle, Washington
March 4-7, 2019
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Background

Latently HIV-infected CD4+T-cells harboring integrated, transcriptionally dormant HIV are a major barrier to cure. Upon antigen encounter, CD4+ T-cells differentiate along a maturation pathway (**Fig 1**) where more differentiated subsets display progressively shorter half lives [1]. CD4+ T-cells nearer the beginning of the developmental spectrum may thus represent privileged sites for long-term viral persistence [2,3] and may define distinct HIV reservoirs [2]. Our understanding of within-host HIV proviral diversity and dynamics in different CD4+ T-cell subsets, however, remains incomplete.

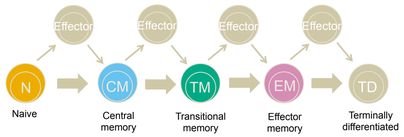


Figure 1. CD4+ T-cell differentiation pathway

Objective

To characterize latent HIV sequence diversity, genetic compartmentalization, and estimated age distribution in different CD4+ T-cell subsets in 5 participants with long-term viremia suppression on cART

Hypothesis

That, in individuals with long-term viremia suppression on cART, the longer-lived, less differentiated T-cell subsets will harbor older, more evolutionarily distinct within-host HIV lineages and greater overall genetic diversity compared to the shorter-lived, more differentiated subsets.

Approach

N: Five participants, with a median 9 years pVL suppression on cART (**Table 1**).

CD4+ T-cell subsets: Naïve (N), Central memory (CM), Transitional Memory (TM) and Effector Memory (EM) CD4+ T-cells were sorted from CD4+ T-cells isolated from PBMC using CD3, CD4, CD45RA, CCR7 and CD27 as markers, as described in [2].

Pre-cART plasma sampling: A median 8 [IQR 4-15] pre-cART plasma samples, spanning a median 5 [IQR 1-10] years were used to reconstruct within-host HIV evolutionary histories.

HIV sequencing and analysis: HIV *nef* was amplified from pre-cART plasma HIV RNA and proviral DNA from CD4+ T-cell subsets using single genome approaches. Phylogenies were reconstructed by maximum-likelihood (RAxML; GTR model). Genetic variation between subsets was assessed with analysis of molecular variance (AMOVA) and tree-based measures of phylogenetic diversity were assessed using R. HIV sequence compartmentalization was assessed using the Slatkin-Maddison (SM) test. Proviral ages were estimated using a phylogenetic approach that involves the temporal reconstruction of within-host HIV evolution to infer within-host evolutionary rate information [4].

Table 1: Participant clinical and sampling characteristics

Pt	years infected pre-cART	N pre-cART plasma timepoints	Nadir CD4 count	Years cART suppressed
1	10	14	1130	9
2	11	16	780	9
3	5	8	650	8
4	2	5	460	9
5	14	2	650	16

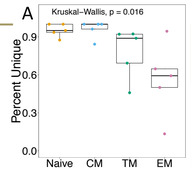
Table 2: Number of sequences collected

Pt	Total pre-cART plasma HIV sequences	Latent HIV sequences: Naïve N (intact)	Latent HIV sequences: CM N (intact)	Latent HIV sequences: TM N (intact)	Latent HIV sequences: EM N (intact)	Total (intact)
1	102	52 (31)	33 (26)	33 (26)	30 (27)	**250 (212)**
2	147	20 (20)	16 (13)	19 (18)	15 (15)	**217 (213)**
3	122	35 (16)	24 (19)	48 (38)	26 (18)	**255 (213)**
4	72	18 (16)	22 (20)	29 (26)	19 (18)	**160 (152)**
5	42	31 (20)	23 (16)	24 (24)	22 (17)	**142 (119)**

Results Summary

Latent HIV diversity differed between hosts, and in some cases within CD4+ T-cell subsets in a host. Notably:

- **EM harbored the highest frequency of identical HIV sequences**, consistent with clonally-expanded HIV populations in this subset (**Fig 2A**).
- 3/5 participants exhibited evidence of genetic compartmentalization between CD4+ subsets (**Fig 2B**).
- **Inferred proviral ages and/or root-to-tip distances differed between CD4+ T-cell subsets in 2 participants, though not in consistent directions** (**Fig 3D, Fig 8C**).
- There was no difference in the phylogenetic diversity nor distinctiveness of the subsets across participants (**Fig 2C, D**).



B

Pt	Slatkin-Maddison p-value	AMOVA p-value	Is latent HIV in CD4+ T-cell subsets compart-mentalized?
1	0.025	0.006	YES
2	0.039	0.003	YES
3	0.06	0.147	NO
4	0.167	0.123	NO
5	<0.001	<0.001	YES

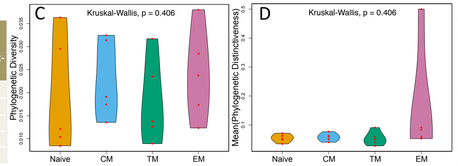


Figure 2. Assessing latent HIV genetic distribution in CD4+ T-cell subsets. *(A)* Identical HIV sequences are most often found in EM *(B)* Latent HIV populations exhibited genetic compartmentalization in 3/5 participants. *(C)* In an analysis across participants, there was no difference in phylogenetic diversity or *(D)* phylogenetic distinctiveness between HIV sequences in different CD4+ T-cell subsets

Within-host latent HIV diversity and age estimates

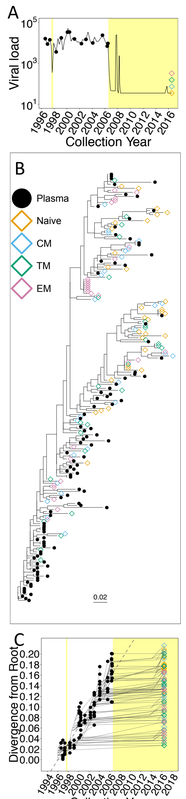


Figure 3. Participant 1. (*A*) Plasma HIV RNA sequences from 14 pre-cART time points spanning August 1996 to June 2006 were used as training data (black circles) to infer the integration dates of proviral sequences sampled from CD4+ T-cell subsets in 2016 (colored diamonds). (*B*) Rooted tree (by root-to-tip regression) relating proviral and pre-cART plasma sequences (C) Linear model (gray dotted diagonal) relating HIV sequence divergence from the root to time, with ancestor traces overlaid. (D) Inferred integration dates of proviral sequences. **Naïve cells harbor "younger" virus.**

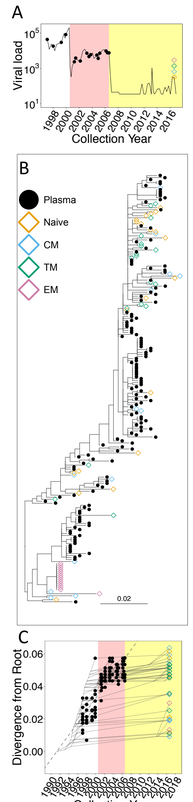


Figure 4. Participant 2. *(A)* Plasma HIV RNA sequences from 4 pre-ART time points between Feb. 1997 and Dec. 1999, and 12 time points between April 2001 and August 2006 during incompletely suppressive dual ART were used as training data (black circles) to infer the integration dates of censored sequences sampled from CD4+ T-cell subsets in 2016. *(B)* Rooted tree (by root-to-tip regression) relating proviral and pre-cART plasma sequences. *(C)* Linear model *(D)* Inferred integration dates of proviral sequences. **No significant difference in sequence age was found between subsets.**

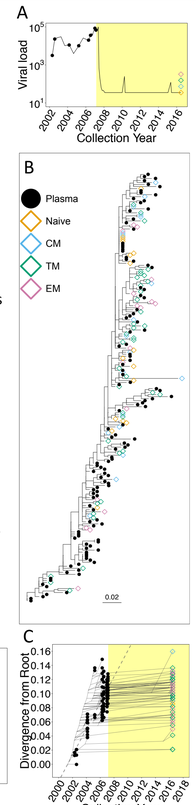


Figure 5. Participant 3. (*A*) Plasma HIV RNA sequences from 8 pre-cART time points spanning April 2002 to March 2007 were used as training data (black circles) to infer the integration dates of censored proviral DNA sequences sampled from different CD4+ T-cell subsets in 2016 (colored diamonds). (*B*) Rooted tree (by root-to-tip regression) relating proviral and pre-cART plasma sequences. (C) Linear model (gray dotted diagonal) with ancestor traces overlaid. (*D*) Inferred integration dates of proviral sequences. **No significant difference in sequence age was found between subsets.**

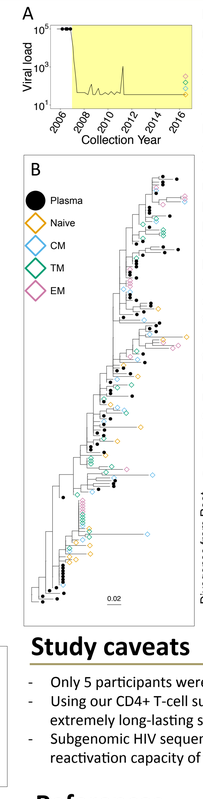


Figure 6. Participant 4. (*A*) Plasma HIV RNA sequences from 5 pre-cART time points spanning Sept. 2005 to Jan 2007 and proviral DNA sequences from T-cell subsets sampled in 2016. (*B*) Outgroup-rooted tree relating proviral and pre-cART plasma sequences. Outgroup rooting was performed as a conservative way to estimate the root due to the limited pre-cART follow-up, though root-to tip regression returned comparable results. *(C)* **HIV sequence divergence from the root did not differ between T-cell subsets.**

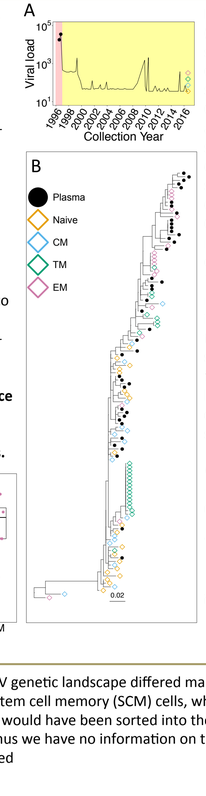


Figure 7. Participant 5. (*A*) Plasma HIV RNA sequences from 2 pre-cART time points spanning July 1996 to Oct. 1996 (black circles) and the proviral DNA sequences sampled from different CD4+ T-cell subsets in 2016 (colored diamonds). (*B*) Outgroup-rooted tree relating proviral and pre-cART plasma sequences. Root-to-tip rooting was not possible due to insufficient pre-cART timepoints *(C)* **HIV sequence divergence from the root was higher in more differentiated T-cell subsets.**

Study caveats

- Only 5 participants were studied, and latent HIV genetic landscape differed markedly between them
- Using our CD4+ T-cell subset sorting strategy, stem cell memory (SCM) cells, which may serve as an extremely long-lasting site for HIV persistence, would have been sorted into the naïve compartment.
- Subgenomic HIV sequencing was performed, thus we have no information on the integrity or reactivation capacity of the HIV genomes studied

Discussion & Conclusions

We found multiple cases of identical sequences existing across different CD4+ T-cell subsets, consistent with latently-infected precursor cells giving rise to more differentiated progeny harboring the same HIV genome. Identical sequences within the same subset, consistent with clonal expansion, occurred most often in TM and EM. Latent HIV genetic composition in CD4+ T-cell subsets may be more complex than previously thought. In all participants, latent HIV sequences were phylogenetically well-intermixed, with 2/5 participants showing no evidence of compartmentalization whatsoever. **Importantly, summarizing across participants we found that, in contrast to our hypothesis, latent HIV sequences in different CD4+ T-cell subsets displayed no consistent patterns with respect to phylogenetic diversity, phylogenetic distinctiveness, nor inferred sequence ages/divergence from root**. Nevertheless, in all participants for whom substantial pre-cART sampling history was available, latent HIV genomes interspersed throughout the within-host phylogeny, consistent with the reservoir as an archive of within-host evolutionary history [4].

References

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2. Chomont et al, *Nat Med* 2009; 15: 893-901
3. Buzon et al, *Nat Med* 2014; 20: 139-144
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