Identical sequence expansions are predominantly found in effector memory T cells

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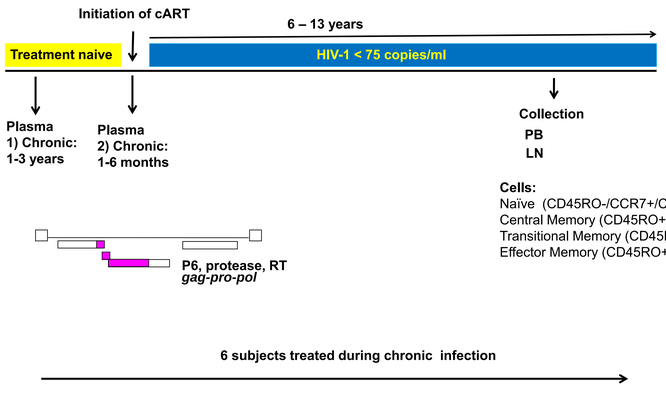
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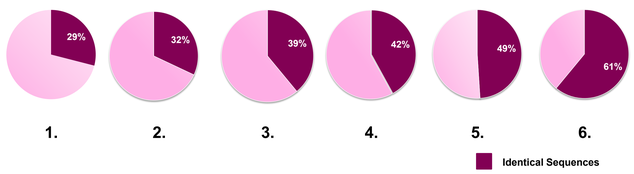
Background:

The effects of cellular proliferation, differentiation and expansion on the maintenance and genetic composition of HIV-1 populations within specific T cells is uncertain.

To address this issue, we examined the distribution of identical HIV-1 intracellular sequences from peripheral blood and lymph node tissue in order to define the role of cell proliferation as a cause of persistence.

Layout of the study:





Results 1

Phylogenetic analyses revealed that 30-60% of all HIV-1 sequences combined from the cells sorted from blood and lymph node tissue (n=93-243 per person) were clonal in nature, and that the mean number of clonal populations per subject was 14 (range 9 to 26). However, only a few (n=0-4) of these clonal sequences were found in pre-therapy plasma sequences.

Table 1. Estimated rate of identical sequences (*gag-pol*) in patients treated during chronic infection: Peripheral blood and lymph node tissue.

Cell Type	1	2	3	4	5	6	7	8	9	10	11	12
	Subject 1		Subject 2		Subject 3		Subject 4		Subject 5		Subject 6	
	Estimated Rate of Identical Sequences, %	95% CI	Estimated Rate of Identical Sequences, %	95% CI	Estimated Rate of Identical Sequences, %	95% CI	Estimated Rate of Identical Sequences, %	95% CI	Estimated Rate of Identical Sequences, %	95% CI	Estimated Rate of Identical Sequences, %	95% CI
Peripheral Blood												
Naïve	19.4	8.2, 35.4	7.7	1.3, 21.9	17.6	4.7, 39.6	0	0.0, 23.8	46.7	23.5, 70.9	50	19.1, 80.9
Central Memory	12.5	3.3, 29.3	8	1.4, 22.7	25	9.8, 46.2	23.1	9.9, 41.3	12.5	2.2, 33.8	23.8	9.3, 44.4
Transitional Memory	25	8.5, 48.9	82.1	65.5, 93.2	28.6	12.5, 49.6	43.8	21.8, 67.6	38.9	19.0, 61.8	69.2	42.3, 89.3
Effector Memory	47.6	27.5, 68.3	67.7	50.3, 82.3	58.3	38.5, 76.5	73.9	54.1, 88.7	55.6	37.0, 73.1	88.2	67.9, 97.9
Lymph Node Tissue												
Naïve	23.3	10.8, 40.3	0	0.0, 10.5	71.4	35.0, 94.6	N/A	N/A	52.9	30.1, 75.0	100	100, 100
Central Memory	13.6	3.6, 31.7	0	0.0, 11.3	42.9	23.4, 63.9	33.3	6.5, 71.9	35.7	14.6, 61.7	80	59.5, 93.3
Transitional Memory	31.8	15.1, 52.5	41.7	23.5, 61.5	36.4	13.0, 65.4	100	100.0, 100	63.2	40.8, 82.2	71.4	35.0, 94.6
Effector Memory	55.2	37.2, 72.3	20	1.3, 62.8	50	21.8, 78.2	50	23.8, 76.2	64.7	41.1, 84.2	33.3	6.5, 71.9

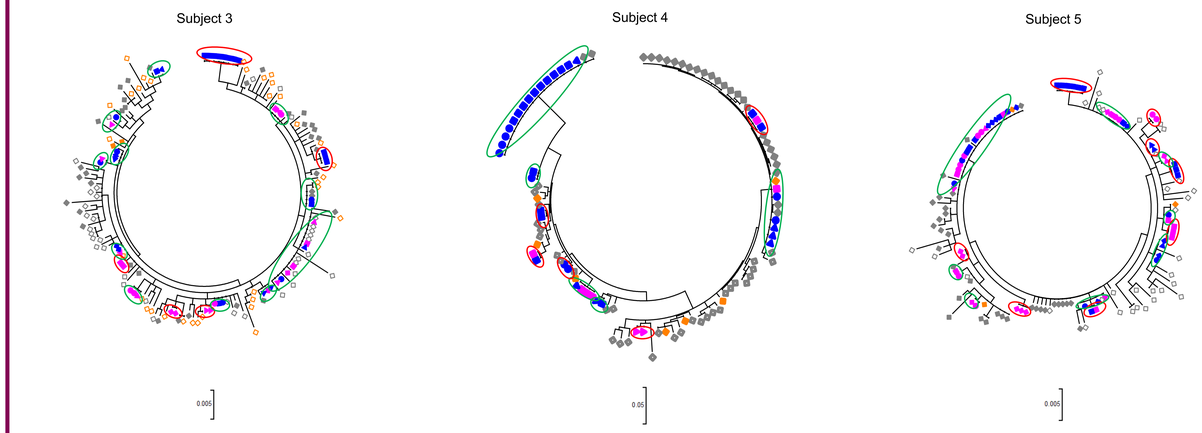


Figure 1. Maximum likelihood trees of pre-therapy sequences from plasma, on-therapy sequences from plasma and cellular HIV-1. The trees represent identical sequences from 3 patients who initiated therapy during chronic infection (patients 3-5). Clonal populations of sequences were determined as ≥2 genetically identical sequences among all the viruses analyzed for each subject (N=92-243) and compared to pre-therapy plasma-derived single genomes.

Red circles indicate clonal populations with only one specific cell type.
Green circles indicate clonal populations with different cell types.

Methods:

Using single-proviral sequencing, we isolated intracellular HIV-1 genomes derived from defined subsets of CD4+ T cells (central (CM)-, transitional (TM)-, and effector (EM)-memory) from peripheral blood and lymph node tissue. Samples were collected from six subjects on long-term suppressive therapy (6-13 years) treated during chronic infection. Unique clonal populations of sequences were determined (for p6-RT) as >2 genetically identical sequences among all the viruses analyzed for each subject and we assessed the number of clonal populations found exclusively within a particular cell type. All clonal sequences were compared to pre-therapy plasma-derived single genomes.

Results 2

Clonal populations were more common in EM cells (61%) than other T cell subsets (25-49%). Across all subjects, the percentage of sequences in EM cells that were clonal sequences and found in no other cell types averaged 30%, whereas this average was <7% for CM or TM cells (both p=<0.0001 versus EM cells). The rates of such clonal populations associated with CM versus TM cells were not significantly different (p=0.21).

Conclusions:

Sequences in EM cells were predominantly clonal. These clones were often not found in less differentiated cells. The source of these cells is not known, but may reflect infection and expansion directly of an infected EM population, or differentiation and expansion of infected progenitor cells in tissue. This suggests that different cellular mechanisms are contributing to the persistence of HIV within different memory T cell subsets and the size and distribution of the latent reservoir during suppressive therapy is likely to be shaped in part by proliferation and expansion of differentiated T cells.

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