HIV Drug Resistance Program
NCI-Frederick

Massive Expansion of HIV Infected Cells with Identical Proviruses in Patients on Suppressive ART

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BACKGROUND

To investigate the mechanisms for the emergence of identical sequences on ART, we identified 14 patients with clonal HIV sequences during long-term therapy and determined their relationship to CD4+ T cell count and HIV DNA copy number.

METHODS

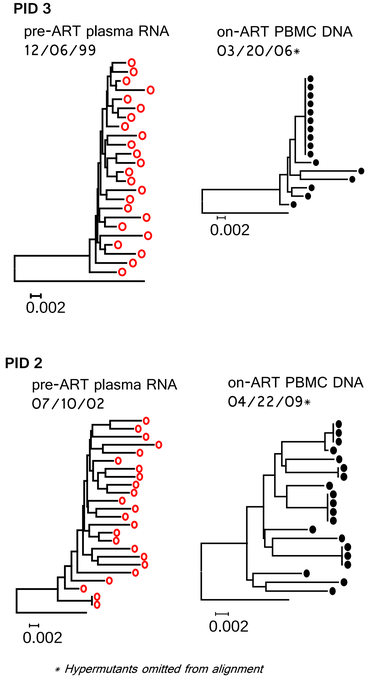
Single-genome *pro-pol* sequences from PBMCs were obtained from 14 subtype B-infected patients at the time of initiating ART and during suppression for 4-12 years. Clusters of identical sequences were identified and the proportions of identical sequences were plotted against baseline CD4+ T cell count and HIV DNA copy number and change in CD4+T cell count on ART. Estimates for the total number of CD4+ T cells with clonal proviruses were determined by multiplying the fraction of identical sequences by the normalized HIV DNA copy number per million CD4+ T cells and the estimated number of total body CD4+ T cells.

CONCLUSIONS

Analyses of the genetics of HIV proviruses during ART suggests a relationship between the total DNA copy number pre-ART and the change in the CD4+ T cell count on ART with the emergence of identical sequences during therapy in those patients initiating ART with very low CD4+ T cell copy number. This finding suggests that increasing numbers of CD4+ T cells on ART is due, in part, to the expansion of infected cells. Estimates on the number of CD4+ T cells carrying identical HIV proviruses suggests that infected cells expand massively before and during ART generating billions of new infected cells from a single initial infection event despite effective ART.

RESULTS

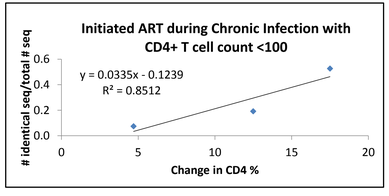
The fraction of identical sequences in PBMCs during long-term ART ranged from 7% to 55% of the total HIV DNA. In 3/13 patients who started therapy during chronic infection with T cell counts <100/ul, there was an apparent positive relationship between the change in CD4+ T cells following ART initiation and the emergence of identical sequences and a negative relationship with the HIV DNA copy number. There was no obvious relationship between the CD4+ T cell count or the HIV DNA copy number in patients who initiated ART during chronic infection with >100 CD4+ T cells/ul or in patients who initiated ART during early infection. Estimates of the total body number of CD4+ T cells carrying clonal HIV sequences ranged from $1.3x10^9$ to $6.9x10^{10}$.

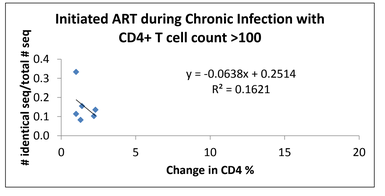


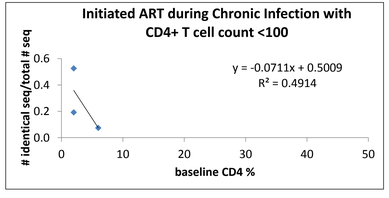
* *Hypermutants omitted from alignment*

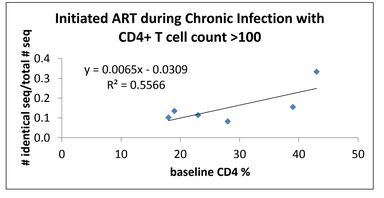
PID	patient description	proportion of identical sequences after long-term ART	DNA copies/CD 4+ cells after long-term ART	CD4+ cells/ul after long-term ART	total cell volume	Estimated # blood cells producing identical sequences	Estimated # CD4+ cells containing identical sequences
1	Initiated ART during chronic infection with CD4+ T cell count **<100**	0.074	658	288	5000	7.01E+07	**3.51E+09**
2		0.191	1046	539	5000	5.40E+08	**2.70E+10**
3		0.526	1071	495	5000	1.39E+09	**6.97E+10**
4	Initiated ART during chronic infection with CD4+ T cell count **>100**	0.333	169	699	5000	1.97E+08	**9.84E+09**
5		0.155	453	1289	5000	4.52E+08	**2.26E+10**
6		0.135	81	493	5000	2.68E+07	**1.34E+09**
7		0.114	246	279	5000	3.90E+07	**1.95E+09**
8		0.102	328	782	5000	1.31E+08	**6.54E+09**
9		0.082	212	674	5000	5.87E+07	**2.93E+09**
10	Initiated ART during early infection	0.548	209	691	5000	3.95E+08	**1.98E+10**
11		0.161	562	404	5000	1.83E+08	**9.13E+09**
12		0.423	131	484	5000	1.34E+08	**6.69E+09**
13		0.429	329	562	5000	3.96E+08	**1.98E+10**

PID	duration of HIV infection at start of ART	baseline CD4 %	Change in CD4 %	total # identical/ total seq	chronic <100 vs. chronic >100	chronic vs. early
1	Chronic infection; CD4+ T cell count <100	6	4.7	0.074	p=0.033	
2		2	12.5	0.191		
3		2	17.5	0.526		
4	Chronic infection; CD4+ T cell count >100	43	1	0.333		p=0.05
5		39	1.4	0.155		
6		19	2.3	0.135		
7		23	1	0.114		
8		18	2.2	0.102		
9		28	1.3	0.082		
10	Early infection	28	1.7	0.548		
11		17	1.7	0.161		
12		23	1.8	0.423		
13		30	1.6	0.429		









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