Quantitative and Phylogenetic Analyses of Persistent HIV in Blood and GALT During Long-Term cART

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BACKGROUND

HIV infection results in profound effects on gut associated lymphoid tissue (GALT), and combination antiretroviral therapy (cART) only partly reverses HIV-induced destruction. The role of GALT in ongoing HIV persistence remains uncertain. Previous studies have analyzed HIV sequences obtained from pools of multiple pinch biopsies; the degree of sampling variation in individual biopsies is unknown, but pooling may introduce significant bias in estimating the size and structure of HIV populations. To quantify the distribution of infected cells, and determine the phylogeny of local HIV populations within the gut mucosa, we analyzed endoscopy-derived material as individual or small pools of biopsies. We compared viral populations in gut to those obtained from contemporaneous and pre-therapy plasma and peripheral blood mononuclear cells (PBMCs).

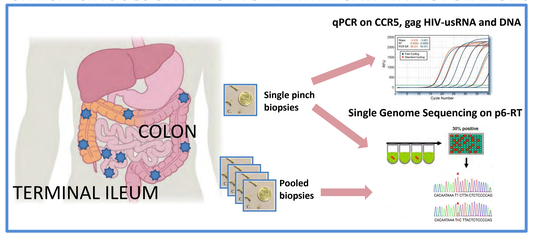
PARTICIPANTS

Pt	Age (y)	Sex	Time since Dx (y)	CD4 nadir (%)	Baseline HIV RNA (cp/ml)	Duration ARV (y)	Duration < 50 c/ml (y)	ARV regimen	CD4 count (%)	Plasma HIV RNA (cp/ml)	Prior evidence of drug resistance
1	46	M	13	364 (19)	27000	12	12.7	TDF/FTC/EFV	616 (51)	<40	no
2	60	M	14	245 (17)	654577	13	12.5	ABC/3TC/NV	404 (29)	<50	no
3	60	M	16	242 (25)	213349	16	15.1	TDF/FTC/RPV	560 (42)	<40	no
4	56	M	24	8 (1)	284700	18	7.3	TDF/FTC/ ATV/rtv	502 (32)	<40	yes
5	64	M	24	180	19000	23	10.4	TDF/FTC/ ATV/rtv/RAL	543 (35)	<40	yes
6	60	M	25	164	1400000	23	5.2	TDF/FTC/RAL	1009 (46)	<40	yes
7	58	M	26	250 (16)	106300	25	2.5	TDF/FTC/EFV	510 (25)	<40	yes
8	62	M	22	56	76000	22	5.4	TDF/FTC/EFV	427 (35)	<40	no

METHODS

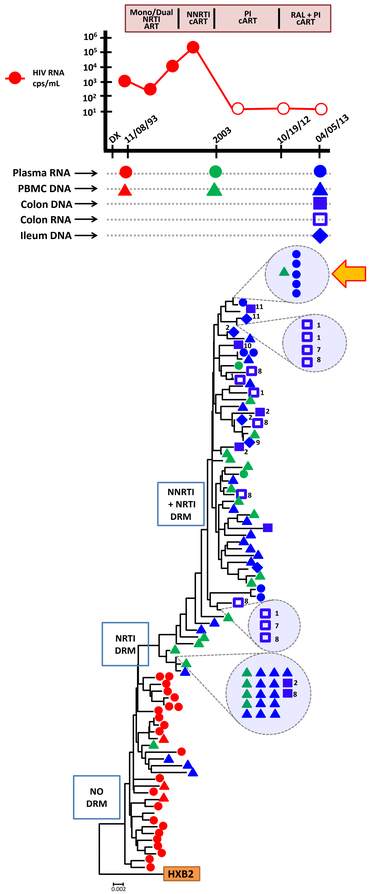
Eight HIV infected patients undergoing antiretroviral therapy and with viral RNA <50 cps/ml for 2.5-10 years were enrolled. Plasma and PBMCs were obtained prior to cART and at time of colonoscopy; small jaws forceps were used to obtain random biopsies (c. 2 mm X 3 mm X 2 mm) from colon and from ileum, when possible. Biopsies were collected as individual snips or pooled in groups of 2-8 snips. Ultrasensitive qPCR assay targeting *gag* was performed to quantify HIV-DNA and RNA copy number; total cell number was determined by qPCR of CCR5 gene. Single genome sequences (SGS) from plasma RNA, PBMC-derived DNA, and gut-derived DNA and RNA were obtained and underwent phylogenetic analysis (MEGA 6, neighbor joining with 1000 of bootstrap).

SITE SPECIFIC SGS STRATEGY TO ANALYZE GALT HIV POPULATIONS



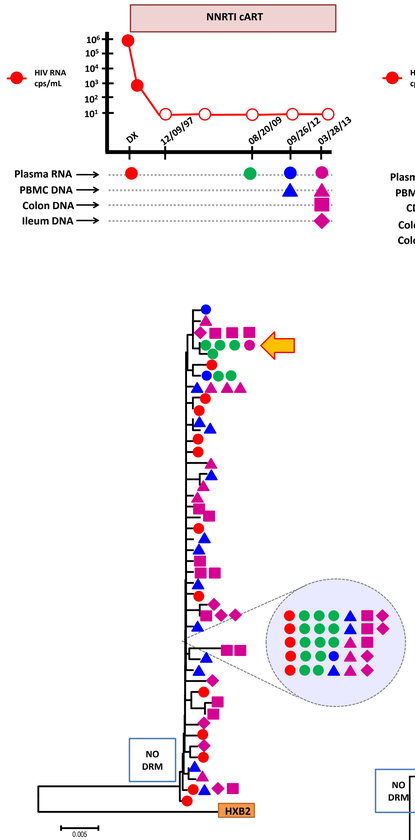
Clonal HIV Populations Are Not Anatomically Restricted in GALT

Pt 5: HIV Suppression after Prolonged Suboptimal Therapy



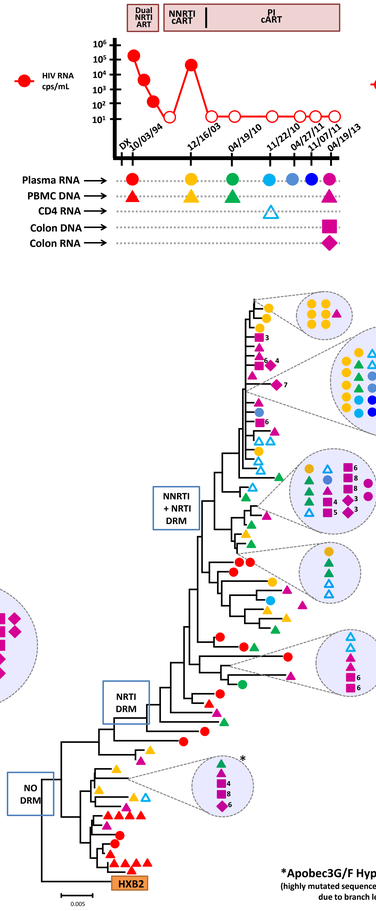
Distinct pinches were numbered ( ). Multiple identical proviruses were found in distant pinches and PBMCs. Expanded clones in DNA did not match the persistent clone in plasma ( ).

Pt 3: Prolonged HIV Suppression After Early cART



Initiation of cART during early infection didn't allow for accumulation of diversity. No divergence was observed between pre-therapy virus and suppressed time points (Distance= 0.002).

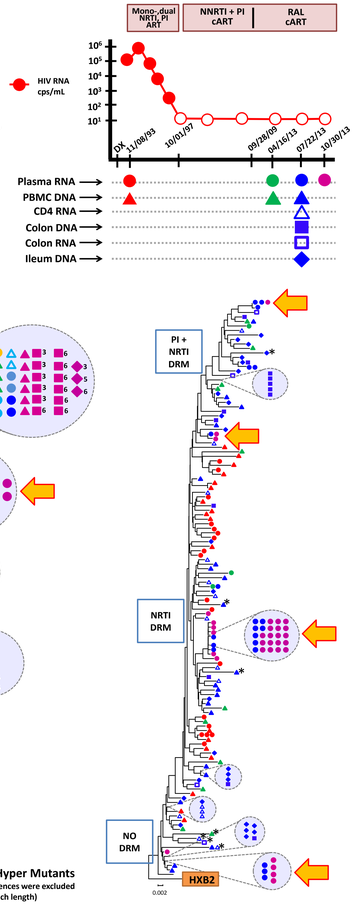
Pt 4: HIV Suppression Following Viral Rebound



*Apobec3G/F Hyper Mutants
(highly mutated sequences were excluded due to branch length)

Distinct pinches were numbered ( ). Multiple identical proviruses were found in distant pinches and PBMCs. One of the expanded clones matches the sequences in gut derived HIV-RNA and HIV-RNA in plasma ( ).

Pt 6: HIV Suppression after Prolonged Suboptimal Therapy



Multiple persistent plasma clones ( ) with distinct drug resistance mutation patterns were found, suggesting residual viremia is coming from different cell clones. No match was found with either gut or PBMCs DNA or RNA.

HIV IS PRESENT IN ALL INDIVIDUAL GUT BIOPSIES

			HIV-DNA			HIV-RNA			
Patient #	Gut Site	Mean CCR5 cell equivalents	Mean copies/10^6 cells	Range	Mean copies per Pinch	Mean copies/10^6 cells	Range	Mean copies per Pinch	Mean RNA/DNA ratio
4	Ileum	na	na	na	na	na	na	na	na
	Colon	9.11E+06	44	21-114	364	23	8-36	205	0.67
5	Ileum	3.67E+06	314	230-510	1102	69	<12-190	246	0.20
	Colon	4.63E+06	206	92-440	928	131	10-220	545	0.67
6	Ileum	3.35E+06	96	66-122	303	56	29-96	161	0.55
	Colon	3.49E+06	98	73-134	338	78	21-132	269	0.76
7	Ileum	3.21E+06	20	15-26	62	21	8-31	80	1.00
	Colon	6.26E+06	37	33-46	230	29	14-51	187	0.85
8	Ileum	5.72E+06	44	19-95	313	52	6-95	377	0.90
	Colon	4.23E+06	21	15-37	98	19	2-58	105	0.81
TOTAL		**4.87E+06**	**128**		**532**	**61**		**265**	**0.61**
	Ileum	**3.86E+06**	**162**		**585**	**54**		**209**	**0.58**
	Colon	**5.68E+06**	**101**		**489**	**65**		**304**	**0.74**

We obtained 6-12 individual pinches from colon in each subject and 6-12 pinches from ileum in 5/8 patients. Single copy detection revealed HIV DNA in all pinch biopsies, with a relatively stable DNA copy number (mean 128 cps/10^6 cells, range 15-510, overall patients). In contrast, HIV RNA levels were more variable and ranged <12-220 HIV RNA cps/10^6 cells. No evidence of compartmentalization was detected, either between gut and peripheral blood or among pinches. Clones of identical sequences were detected in plasma and PBMC and were also present in gut DNA and RNA. Clonal HIV DNA and RNA sequences were identified from multiple snips, suggesting that clones were not anatomically restricted. No genetic divergence from pre-therapy HIV was detected in gut or blood-derived HIV, even after >10y of cART.

SUMMARY

- HIV proviruses are extensively and uniformly distributed throughout the GALT
- An estimated 100 million infected cells present in colonic mucosa despite prolonged cART.
- HIV RNA production varied considerably among pinches. HIV RNA/DNA ratio was significantly higher in colon than in ileum.
- NO divergence of HIV from pre-therapy virus in colon or ileum.
- Clonally expanded populations present in gut mucosa were not anatomically restricted.
- Identical proviruses found both in PBMCs and GALT suggest trafficking of expanded clones.
- Clonal expansion of HIV-DNA isn't reflected in RNA clonal expansion in plasma.
- Detection of the same identical sequences in gut derived DNA, RNA, and plasma suggests that GALT can be a source of persistent viremia on ART.
- Residual viremia can consist of more than one HIV clone, suggesting simultaneous release of virus from different sources, rather than replication.

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NIH Bench to Bedside Program

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