Identifying cellular source of HIV rebound after treatment discontinuation in patients on ART

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Abstract

Objective: To test the hypothesis that peripheral T follicular helper (pTfh) cells are a major cellular compartment of virus persistence in HIV+ patients on combination antiretroviral therapy (cART).

Study Design: Stored PBMC and plasma samples from a unique subset of patients who were in clinical protocols requiring analytic treatment interruption (ATI) from ACTG protocol (A371) were used in this study. PBMC was collected before ATI when the pVL <50 copies/ml and plasma was collected soon after the virus rebound following ATI. pTfh and non-pTfh cells were sorted from PBMC samples and were subjected to cloning and sequencing of partial envelopes (C2V4) using cell-associated DNA and RNA. Single genome amplification (SGA) env (C2V4) from rebounding plasma were sequenced directly. All sequences were analyzed for unique sequences and compared.

Results (ongoing): We have completed 4 patient samples so far in which sufficient amount purified pTfh and non-pTfh cells were obtained after cell sorting to perform the cloning and sequencing. In the initial analysis of the 4 patients, env sequences of pTfh cells from one patient showed phylogenetic association with plasma sequence obtained after rebound. In the other three patients, sequences from plasma virus did not show any relationship with pTfh or non-pTfh sequences indicating a non CD4 T central memory cell source for the rebounding virus in these patients. We are currently analysing two more samples in which sufficient number of cells are available for cloning and sequencing.

Significance: Our expectation is that with env deep sequencing we may be able to show the phylogenetic relationship of plasma and pTfh viruses and may able to identify whether pTfh cells could be a source of plasma virus rebound upon ATI at least in some patients.

Background

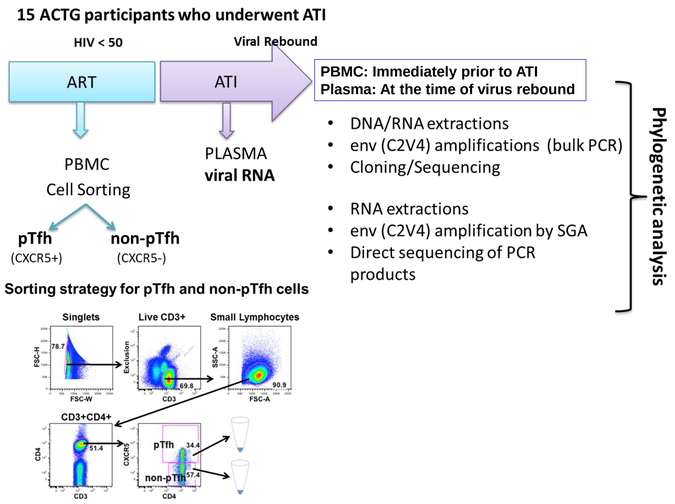
- Tfh cells are specialized memory CD4 T helper cells that are critical for providing help to B cells to generate high affinity Ab[1].
- Tfh cells that reside within the germinal centers in LN are highly permissive for HIV and expand in LN during chronic HIV infection[2].
- In the peripheral blood, a subset of CD4 T_{CM} expressing CXCR5 is important for Ab production. These cells have been termed as peripheral Tfh cells (pTfh) as they share functional properties with Tfh in LN[3].
- We and others have shown that pTfh are highly susceptible to HIV and it has been shown that HIV persists in these cells following plasma viral suppression[4, 5].
- pTfh cells in the circulation represent emigrants from LN and therefore harbor viruses that originate in LN Tfh.

Hypothesis: Tfh are a primary source of virus rebound in plasma if treatment is discontinued

Aim: To compare the HIV env sequences in rebound plasma and pTfh cells in a cohort of HIV infected patients undergoing ATI

Methods

Study Design



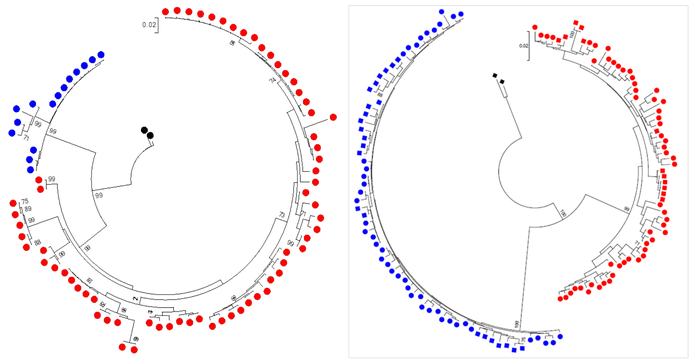
Results

Study Participants and sorted pTfh and non-pTfh cells:

PID	Wk of ART	PBMC used for sorting	pTfh	non-pTfh	ATI VL (copies/ml)	Pre-ATI CD4	Timing of rebound
0250126-G	44	22 M	160,000	400,000	1,054,109	836	29 days
026253-H	44	16 M	105,500	800,000	98,585	1830	54 days
0083102-J	52	20 M	161,000	524,000	1287	546	15 days
0540321-A	52	16 M	280,000	555,000	96	645	13 days
0043656-F	44	20 M	193,000	982,000	1226	704	23 days
0044674-H	44	25 M	154,000	570,000	22,568	878	13 days
0111709-J	47	18 M	66,800	204,000	4,006,954	913	27 days
0520527-L	52	15 M	144,000	366,000	7974	522	41 days
0621388-F	44	6 M	89,000	248,000	10,068	537	36 days
0580020-L	52	18 M	215,000	657,000	6299	617	21 days
0263252-E	40	4 M	36,000	125,000	81,147	867	57 days
0083111-K	44	30 M	350,000	800,000	1317	1520	15 days
0281135-A	48	16 M	127,000	449,000	4748	1099	36 days
0291180-D	44	15 M	367,000	1,047,300	2525	1132	28 days
0111899-K	44	5.5 M	45,889	190,500	4691	852	28 days

Completed Ongoing

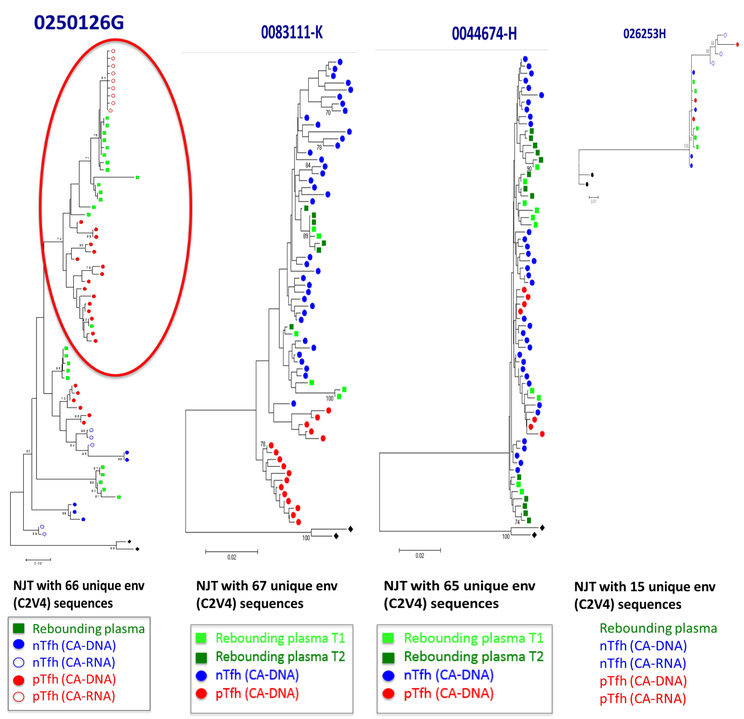
Fig 1: Neighbor-joining tree including env (C2V4) sequences of four patients (126G and 253H) showing no evidence of cross-patient sequence contamination.



Patient 126G - blue
Patient 253H- red

Patient 111K - red
Patient 674H - blue
HIV Ref Strain – black

env sequences of pTfh cells from 1/4 patients show phylogenetic association with plasma sequence obtained after rebound



Summary and Conclusions

1. This study was conducted in a subset of virally suppressed patients (N=15) on ART enrolled in ACTG 371who underwent ATI.
2. Peripheral blood samples were collected immediately prior to ATI and plasma at the time of virus rebound.
3. pTfh and non-pTfh cells were sorted and 6 participants with maximum cell yields were identified for comparison of HIV env sequences in rebound plasma, pTfh and non-pTfh..
4. Of 4 patients analysed to date, env sequences derived from one patient showed phylogenetic association of plasma virus sequence and pTfh cells, suggesting pTfh cells as being a unique source of rebound virus.
5. In the other three patients, sequences from plasma virus did not show any relationship with pTfh or non-pTfh sequences suggesting a non CD4 T central memory cell source for the rebounding virus.
6. The low number of purified pTfh cells from available samples with frequencies of CD4+CXCR5+ pTfh cells at 8-17 % of peripheral blood CD4 T cells was the main limitation of this study. Since patients were on cART, the frequencies of infected cells were likely to be low, resulting in only few unique sequences for conduct of phylogenetic analysis.

Ongoing analysis

- Cloning and sequencing for the remaining two patients (0520527L, 0580020L) in purified cells. SGA and sequencing for plasma.

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